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FORTY YEARS OF SOVIET EXPERIMENTAL MEDICINE AND BIOLOGY

The forty years since that historic event, the Great October Socialist Revolution, have been years of vigorous growth in Soviet experimental medicine and biology.

It is scarcely possible in this article to cover more than the main developments in two such vast divisions of science; the field of experimental medicine is made up of many separate, special branches, encompassing as many problems as the field of clinical medicine. It is therefore impossible in an article of this sort to adequately treat every aspect of these developments. These insufficiencies, however, can be remedied by articles published in other journals which cover a more limited field.

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Before the revolution, there was only one scientific institution in Russia for research in experimental medicine — the Institute of Experimental Medicine in Petersburg — upon which other institutions of this sort were modeled after the revolution. It was the forerunner of the All-Union Institute of Experimental Medicine, which subsequently became part of the USSR Academy of Medical Sciences, and the prototype for certain institutes of the USSR Academy of Science.

However, the universities of Petersburg, Moscow, Kazan, Kharkhov, Kiev, Odessa, Permsk and several other universities carried out work in the region of experimental medicine and biology, as well as the Institute of Experimental Medicine. The Academy of Military Medicine played an important role in the genesis of experimental medicine research. The first experimental medicine research groups began to appear in the Academy of Sciences immediately before the revolution.

These few scientific institutions had the facilities for only limited research. The work, however, was on an extremely high scientific level.

Outstanding workers in experimental biology and medicine, I. M. Sechenov, I. I. Mechnikov, V. O. and A. O. Kovalevsky and K. A. Timiryazev, left the next generation of scientists the beginnings of material understanding of vital phenomena, as well as classic examples of scientific genius.

This unbroken chain of scientific ideas greatly facilitated the continuance of active work by many outstanding scientists in many scientific institutions even after the Great October Socialist Revolution.

Soviet physiology gratefully accepted from the older generation of physiologists and clinical physicians the extremely important conception of neurism, the foundations for which were laid by the works of I. M. Sechenov, S. P. Botkin and I. P. Pavlov. This idea was developed along two main lines: first, by broad and systematic investigation of the physiology of the nervous system, particularly of higher nervous activity, and second, by developing further I. P. Pavlov's classical conception of the idea of neurism, which was as a "physiological course attempting to spread the influence of the nervous system to a potentially greater number of physical activities."

During the post-October period, both of these aspects were developed according to the philosophy of dialectic materialism, and abundant material was obtained confirming the basic principles of the idea.

Higher nervous activity was investigated under the immediate direction of Pavlov until 1936, the year of his death, when his colleagues and students undertook to carry on this study.

During the years following I. P. Pavlov's death, special attention was given to the problem concerning the mechanisms of experimental neuroses and to investigations of cortical inhibition and the formation mechanisms of temporary associations. Work studying higher nervous activity types, the second signal system and the genetics of higher nervous activity was revived after a joint session of the AN SSSR and the AMN SSSR, which pointed out the errors which had been made in the development of the Pavlov scientific legacy.

In works of many outstanding scientific associations headed by P. S. Kupalov, E. A. Asratyan, K. M. Bykov, M. K. Petrova, Yu. V. Folbort, P. K. Anokhin, L. A. Orbeli, A. G. Ivanov-Smolensky, N. A. Rozhansky and many others, the basic rules of higher nervous activity have been examined in detail, as were the mechanisms of conditioned reflex formation, of conditioned association fixation and of neurosis development and successful attempts were made to use a series of new chemotherapeutic substances for experimental therapy in higher nervous activity disturbances. An extremely important achievement was the examination of neuroses in animals under conditions of free behavior. These studies showed that certain American scientists were wrong in asserting that neuroses developed in animals due to their separate confinement. The knowledge obtained regarding neuroses was widely and successfully used, moreover, in clinical practice. On the basis of this knowledge, a new conception of the pathogenesis of hypertonic and ulcerous diseases was introduced and developed (G. F. Lang, A. L. Myasnikov, M. V. Chernorutsky, K. M. Bykov, and I. T. Kurtsin).

The combined efforts of many researchers made serious attempts to understand the nature of inherent cortical inhibition. We are, however, far from solving this "damned question," as I. P. Pavlov called it. This is indicated by the discussion of it at the IX session of the AMN SSSR, which was specially dedicated to the problem of inhibition.

How little we know about it is demonstrated by the fact that there are at least three points of view regarding the mechanism of this process (P. S. Kupalov, I. S. Beritov, and P. K. Anokhin). The original conception, developed by P. K. Anokhin, has been criticized (P. S. Kupalov, 1957) and has not been recognized. Nor is the idea that cortical inhibition is of a parabiotic nature generally accepted (V. S. Rusinov, N. V. Golikov, and others).

Although the phenomenon of nerve link fixation has been the subject of constant research by physiologists for the past 40 years and the general, basic rules of this phenomenon can be considered as established, there is still a wide field for new studies on this subject.

Of great importance was the attempt to understand the complex forms of animal behavior ("situation reflexes"). However, we can now assume that, using the principal patterns established by I. P. Pavlov, the most complex forms of animal behavior can be explained and understood.

The studies which have shown the existence and significance of natural conditioned reflexes which are present in animals living under natural conditions also deserve great credit (A. D. Slonim and colleagues).

Much research has been done in the field concerning the types of higher nervous activity, a field founded by I. P. Pavlov. During the past 40 years, we have established new variations of the types, and new methods of procedure have been developed which make it possible to determine the type of higher nervous activity and, which is very important, to show the dependency of the development and duration of many pathologic processes on the typological group of the animal (A. G. Ivanov-Smolensky and others). Such studies have aided the practical use of knowledge concerning higher nervous activity types, which, in many cases, made possible the development of differentiated methods of treatment.

Recently, physiologists and physicians have been much occupied with the question of the protective and curative role of inhibition, and medicamental sleep has been widely used both experimentally and clinically (E. A. Asratyan, A. G. Ivanov-Smolensky, A. L. Myasnikov, A. A. Vishnevsky, N. V. Konovalov, and others). Although errors have been allowed in a series of cases and the use of sleep therapy for certain diseases is insufficiently substantiated, this type of scientifically grounded therapy has been definitely accepted for practical use. During the forty-year period, still another aspect of higher nervous activity has been successfully investigated, namely the central nervous system's actual role in compensating for damage and its compensating role in the development of pathologic processes (A. D. Speransky, E. A. Asratyan, P. K. Anokhin and colleagues). Research on the phenomena of nervous system plasticity and on certain general patterns of disturbed function compensation has made possible the extensive application of purely physiological aspects to practical, clinical problems and needs, particularly those of neurosurgery.

A particularly important branch of I. P. Pavlov's work was his position regarding the second signal system. I. P. Pavlov conceived the main theories and outlined their further development as a specific problem, which would help show the extent to which the basic principles of animal higher nervous activity apply in human higher nervous activity.

The intensive development of research on the second signal system after the joint session considerably deepened and broadened our knowledge of the characteristics of human higher nervous activity (A. G. Ivanov-Smolensky and colleagues, K. M. Bykov and colleagues, N. I. Krasnogorsky and colleagues). It can now be considered that firm, if rudimentary, foundations have been laid for the comprehension of human higher nervous activity. This aspect of research on higher nervous activity, only the principal outlines of which were suggested by I. P. Pavlov, has been entirely developed over the last 40 years.

Important results have also resulted from the research accomplished in the region of nervous system age physiology (N. I. Krasnogorsky, A. G. Ivanov-Smolensky, A. A. Volokhov, A. A. Markosyan, A. N. Kabanov, N. M. Shchelovanov and colleagues and others).

These studies disclosed the principal features of higher nervous activity in children, as well as the genesis of the second signal system. Many of the results from these studies have been utilized in pediatric practice.

Finally, there is yet another important aspect of higher nervous activity research which did not exist at all forty years ago and has been developed during this forty-year period. We have in mind the use of electroencephalography to examine certain features of higher nervous activity.

These studies developed rapidly after their initiation thirty years ago and soon took definite shape. This research in the Soviet Union was developed by comparing electroencephalographic data with those obtained by the conditioned reflex method. Certain foreign scientists (Gasto) have now adopted this up-to-date method.

Thanks to the works of many scientists (S. A. Sarkisov, L. G. Trofimov, M. N. Livanov, E. A. Zhirmunskaya, V. S. Rusinov, I. S. Beritashvili and colleagues, A. B. Kogan, G. V. Gershuni and colleagues and others), adequate correlations have been established between electroencephalographic changes and higher nervous activity in animals, and valuable new data has been obtained.

Finally, one must observe that scientists working in this field (L. G. Trofimov, O. S. Adriapov, S. Ya. Rabinovich and others) have obtained significant results by comparing electroencephalographic changes with the delicate morphology of the cerebral cortex as well as with higher nervous activity.

The extensive development of the works most characteristic to Soviet physiology, i.e., those on the physiology and pathology of higher nervous activity, has been paralleled by the development of research on the general physiology of the central and peripheral nervous systems. The amount of work accomplished in this field is enormous, and significant progress has been made over this forty-year period.

To begin with, the studies directed toward the development of N. E. Vvedensky's ideas have assumed great importance during the last forty years. His highly talented pupil, the outstanding physiologist A. A. Ukhtomsky, developed research on lability and introduced an extremely important principle of nervous coordination – the principle of the dominant. This principle is very important to the dynamic understanding of Sherrington's laws of nervous coordination. It has been adopted by a wide circle of pathophysiologists and is often used to explain the mechanisms of pathologic processes (A. D. Speransky, A. N. Magnitsky, I. A. Arshavsky and others).

The works of the Leningrad University school of physiologists (N. V. Golikov, E. K. Zhukov, P. O. Makarov, L. V. Latmanizova, Yu. M. Uflyand, L. L. Vasilyev, F. P. Petrov, D. N. Lapitsky, V. E. Delov, I. A. Arshavsky and many others) developed an unusual and original approach to the study of the excitation and inhibition processes involving the extensive use of the parabiotic theory to explain excitation and inhibition processes in all parts of the nervous system. However, certain concepts developed by this school (the notion of the role played by perielectrotonic changes in the activity of the nervous centers and the idea of impulseless signalization) still need extensive experimental verification.

During the years of Soviet rule, neurophysiology has grown and improved, encompassing a wide circle of problems resulting from the study of both the general rules of central nervous system activity and the specific physiology of its different sections. The numerous works of a series of Soviet physiologists (D. S. Vorontsov and

colleagues, I. S. Beritashvili and colleagues, L. A. Orbeli and colleagues, P. A. Anokhin and colleagues, N. A. Rozhansky, E. A. Asratyan and colleagues, N. F. Popov and colleagues, N. A. Yudenich, F. N. Serkov, D. G. Krasov, P. E. Motsny, D. M. Gedevani and others) have investigated specific physiological problems regarding various sections of the nervous system and suggested general conclusions from the results obtained.

Specifically, we should like to mention the development of the physiology of the cerebellum and the study of how this section of the central nervous system influences the vegetative processes, also, the study of the dynamic localization of functions in the spinal cord and the theory developed by I. S. Beritashvili concerning the neuronal-neurophilic structure of the nervous system. Although I. S. Beritashvili's views have been subject to criticism, an attempt to comprehend the function of the central nervous system as a whole cannot be ignored. The point is that, as early as 1936 and 1938, I. S. Beritashvili introduced the idea concerning the regulating significance of the reticular formation, anticipating subsequent studies in this direction. Note should be taken of P. K. Anokhin's studies on the interrelations of the center and periphery, as they enabled him to introduce a series of original theories regarding the mechanisms of integration.

The important works of E. A. Asratyan on the dynamic localization of functions in the spinal cord and describing the consequences of total resection of the cerebral cortex are well and widely known.

Finally, we wish to mention the extensive research which has been done (L. A. Orbeli and colleagues) on the physiology of the sympathetic nervous system. The adaptation-trophic theory of sympathetic nervous system activity has been very important to the comprehension of the functions of this section of the nervous system. The data obtained by L. A. Orbeli's school is particularly important now in the light of new data obtained by foreign scientists showing the efferent influences on the function of the receptors.

L. A. Orbeli's development of the evolutionary approach to the analysis of physiological processes and the work done by K. M. Bykov on the problem of the functional interrelations between the cerebral cortex and internal organs are among the most outstanding theoretical contributions to this past period.

The well-known works of K. M. Bykov and his colleagues, continuing and developing the ideas of I. M. Sechenov and I. P. Pavlov, have created a new chapter in contemporary physiology, closely associated with contemporary practical medicine. The works of K. M. Bykov have demonstrated the influence of the cerebral cortex on the condition of the body viscera (and therefore, how the internal condition is affected by the outer world) and also how cerebral cortex activity is influenced by the internal condition of the body. This established a firm foundation for the clinical study of vegetative neuroses and made it possible to suggest the corticovisceral theory in a series of illnesses. Although this theory cannot be considered to be a universal "theory of medicine," in many cases it is very useful in understanding the pathogenesis of certain diseases and can help in their treatment.

Among the outstanding workers in neurophysiology, A. F. Samoilov and N. A. Mislavsky must be gratefully named. The brilliant experimentor, A. F. Samoilov, made an important contribution to research on spinal cord coordinations, and N. A. Mislavsky, who founded the extensive school of physiologists in Kazan, enriched science with magnificent studies on the vegetative nervous system and on nervous regulation of vascular tonicity.

We should mention that A. F. Samoilov was one of the first to express the idea that stimulation in neuromuscular synapses is chemically transmitted.

The studies of L. S. Shtern and her colleagues have contributed much to research on the stability of internal body conditions. Although these works contained some exaggerations (which have been criticized) in their development of ideas concerning the hemato-encephalic and histohematic barriers, they did play a definite part in the development of our opinions on nervous system nourishment and indicate new methods of directed action on its function.

Much has been done during this period toward the formulation of a general theory of stimulation. As well as the above-mentioned achievements of the Vvedensky-Ukhtomsky school, there have been attempts to discover the very nature of the excitation process (D. N. Nasonov, Kh. S. Koshtoyants). The enzymo-chemical theory of excitation introduced by Kh. S. Koshtoyants was valuable as an attempt to connect physiological and biochemical processes.

The brilliant studies of D. N. Nasonov, V. Ya. Aleksandrova and their colleagues A. S. Troshin and B. P. Ushakov deserve special note as they developed and proved a new, denaturation theory of stimulation. The importance of these studies should be especially clear from the fact that this theory is successfully competing with the well-known membranous theory of excitation, almost universally accepted abroad.

Finally, an equally important role in the development of ideas concerning the transmittance of nervous excitation was played by the studies of a group of Soviet scientists on mediators of nervous excitation (L. A. Orbeli, K. M. Bykov, A. V. Kibyakov, A. G. Ginetsinsky, I. P. Razenkov and colleagues and M. Ya. Mikhelson).

During the past forty years, the physiology of the sensory organs has been extensively studied in Soviet physiology. There was practically no research in this important field in Russia before the Great October Socialist Revolution, if one excludes the studies and famous traditions left by I. M. Sechenov. Thanks to the combined efforts of many researchers (L. A. Orbeli, L. A. Andreev, S. V. Kravkov, G. V. Gershuni, P. O. Makarov, N. I. Grashchenkov and colleagues, P. G. Snyakin, A. V. Lebedinsky), important information on the physiology of the sensory organs has been obtained. The sciences of physiologic optics and acoustics, which are now separate and independent divisions of physiology in our country, are based on these studies.

Important progress has also been made during the last 40 years in the development of ideas concerning the nervous regulation of functions. The length of this paper does not permit us to summarize here the enormous number of works done in this field during the past 40 years.

The systematic study of the physiology of digestion has become a tradition of Soviet physiology. During this forty-year period, much important data has been accumulated on the nervous regulation of digestive tract activity (K. M. Bykov and colleagues, I. T. Kurtsin and colleagues, A. N. Bakuradze and colleagues, G. V. Folbort and colleagues), especially data regarding cortical influences; valuable information has also been obtained on the physiology of human digestion by means of observations on people with operative fistula. K. M. Bykov's theory on the gastric secretory fields has found clinical approval.

The broadly conceived and successful studies of I. P. Razenkov and a group of his colleagues have enriched our knowledge of digestive changes caused by lowered barometric pressure, and I. P. Razenkov's significant monograph, "New Data on the Physiology and Pathology of Digestion" raised and solved many questions on neuro-humoral regulation of digestive tract motor and secretory activity.

These studies, which were conducted in close association with the clinic, found the answer to a series of clinical problems and should be regarded as a definite chapter in the study of the physiology and pathology of digestion.

We should also mention the important results obtained from research on metabolism and on the physiology of nutrition (O. P. Molchanova, G. K. Shlygin and colleagues). The works of this collective made possible the scientific development of a series of medicinal diets and regimes.

The famous legacy in the study of nervous regulation of circulation left to us by I. P. Pavlov has been developed considerably in the past forty years (A. I. Smirnov, V. V. Parin, A. P. Polosukhin, N. A. Mislavsky, G. P. Konradi, M. E. Marshak, K. M. Bykov, M. G. Udelnov, I. N. Davydov, P. M. Starkov). These studies furnished new and valuable facts on nervous regulation of heart activity and made it possible to study cortical regulation of cardiac and vascular activity, to solve the problem regarding the reflex autoregulation of circulation both as a whole and in separate vascular regions (brain, heart), as well as to obtain important data concerning the vasomotor regulation of individual organs.

V. N. Chernigovsky and his colleagues studied in detail the influences of the various reflexogenic zones on circulation, respiration, the blood system and the locomotive functions; they established the basic pattern of these reactions, made an electrophysiologic analysis of the afferent impulses from the interoceptors and showed the corticocerebral zones related to the latter.

We wish to mention, however, that research on the problem of "pure" hemodynamics has not made much progress due to the greater attention given by physiologists to the problem of nervous regulation of circulation. Only recently have successful attempts been made to work out this problem systematically (V. V. Parin, E. B. Babsky).

Soviet physiologists have made definite progress in the study of respiration regulation. Thanks to the efforts of various collectives directed by M. V. Sergievsky, M. E. Marshak, L. L. Shik and others, valuable data have been obtained on the physiology of human respiration during labor and athletic activity. M. V. Sergievsky discovered important facts concerning central regulation of respiration and studied in detail the activity of the respiration center.

Valuable data on the condition of the respiration in patients from whom part or all of a lung had been removed were obtained by P. K. Anokhin and his colleagues.

Proven results were obtained by Soviet physiologists from research on the nervous regulation of excretion. The works of many researchers (G. M. Shpuga and colleagues, K. M. Bykov and colleagues, L. A. Orbeli and colleagues, etc.) examined the mechanisms of nervous and hormonal regulation of kidney activity. These studies made it possible to explain many features of kidney participation in the general system of neuro-humoral body regulations. Some attention has also been given to the problems involved in the physiology of urine excretion. In this context, we note the extremely important work conducted by A. G. Ginetsinsky and colleagues, who are progressively and successfully developing this difficult division of physiology.

There is still another aspect of physiology which has been developed during the past forty years – age physiology. Through the efforts of several scientists in this field (I. A. Arshavsky, A. A. Volokhov, L. A. Orbeli and colleagues, A. G. Ginetsinsky, A. A. Markosyan, N. I. Kasatkin and others), the ontogenic formation of the motor apparatus and that of several vegetative functions have been successfully traced.

These valuable studies established scientific facts on which physical education and hygienic measures could be based.

Finally, during the years of Soviet rule, the physiology of sport and labor has been developed through research (D. Slonim, V. S. Farfel, N. K. Vereshchagin, G. P. Konradi, S. A. Kosilov, N. V. Zimkin, K. M. Smirnov and others). However, although knowledge of the physiology of sport has advanced considerably, the progress in the study of labor physiology leaves much to be desired.

During the years of Soviet power, active research work by physiologist collectives was instituted in the national republics and is now being carried on. In Uzbekistan, a large collective of physiologists are actively developing the studies of A. Yu. Yunosov. A. I. Karaev and a group of associates are conducting extremely vital work in Azerbaidzhan. The work of Turkmen's A. I. Venchikov in electrogastrography is well known. In Kazakhstan, there is A. P. Polosukhin, who founded and trained large research collectives and published many valuable studies on age physiology and on the physiology of the lymphatic system. V. L. Lashas is directing the energetic development of research in Lithuania, as E. G. Kyaer-Kingisep is doing in Estonia. In the Tatar Republic, where such outstanding physiologists as N. A. Mislavsky and A. F. Samoilov worked, much research has been done on the chemical transmittance of nervous impulses under the direction of A. V. Kibyakov. In Kirgiz, A. D. Slonim, G. P. Konradi and S. M. Dionesov have made great contributions to the development of physiologic research.

In Belorussia, work has been energetically developed by A. A. Zubkov, I. A. Vetokhin and I. A. Bulygin, who heads the USSR Academy of Sciences Institute of Physiology. A. A. Zubkov has done much work in Moldavia, and, in Armenia, a group of physiologists (R. Kh. Bunyatyan, G. P. Oganesyan and many others) are engaged in investigating problems of higher nervous activity, as well as of nervous system physiology and cortico-visceral physiology. In Latvia, research on hemodynamics is being directed by N. V. Danilov, who was largely responsible for training Uzbekistan's national cadre of physiologists.

In Bashkir, V. V. Petrovsky is conducting effective research on the physiology of the lymphatic system.

In the Ukraine, I. P. Pavlov's pupil, G. V. Folbort, and A. M. Vorobyev, whose untimely death we mourn, have turned out a massive work on the physiology of digestion.

Of course, in a brief summary such as this, we cannot begin to cover the mass of research which has been carried out and the many separate problems which have been solved by Soviet physiologists during this period. Due to the wide range of research and the ever increasing number of physiologists, physiology will continue to progress in the Soviet Union.

In summarizing the development of physiology, we have involuntarily touched upon certain questions of pathologic physiology. This shows the close association of these two branches and their genetic affinity.

Actually, one of the first pathophysiologists in pre-revolutionary Russia, V. V. Pashutin, was a student of I. M. Sechenov. I. P. Pavlov also left us brilliant examples of work in pathology and founded a new branch of pathophysiology – the pathology of higher nervous activity.

The general pathology set forth in the works of V. V. Podvysotsky, L. A. Tarasevich, A. I. Talyantsev and A. B. Fokhta definitely influenced the works and research of Soviet pathophysiologists active in the post-revolutionary period.

Until recently, pathologic physiology was only taught as a separate and specific branch of science in the USSR. In other countries, this branch was combined with either physiology or pathologic anatomy.

During the years of Soviet power, certain outstanding original lines of work were initiated, broadly developing various problems of pathologic physiology.

The ideas of neurism found brilliant and original expression in the works of Academician A. D. Speransky, his students and followers.

His study of the nervous system's role in the development of infectious processes, his extensive development of the question of nerve trophics and his analysis of the dystrophies have placed A. D. Speransky in the front rank of world scientists developing questions of general pathology. His conception of the correlations between the specific and nonspecific effects of stimuli was of great practical use. A scientific basis was established for methods of nonspecific therapy, some of which have long been in practical use.

Ideas introduced by A. D. Speransky made possible the re-evaluation of the part played by the nervous system in body immunology and reactivity. His theories on the mechanisms of disease, convalescence and health and on subsequent reactions are an outstanding contribution to the science of disease and have brought him great fame here and abroad.

The research directed by N. N. Anichkov deserves an honorable place in the development of Soviet pathophysiology. He introduced and perfected the contemporary conception of atherosclerosis – an outstanding contribution to science. The experimental resolution of the atherosclerosis problem made it possible to evolve the practical prophylactics, treatment and diagnosis of this disease (G. F. Lang and colleagues, A. L. Myasnikov and colleagues). N. N. Anichkov obtained interesting data from a study on the problem of auto-infection.

I. P. Petrov, P. N. Veselkin and P. P. Goncharov have done important work on the pathology of circulation, the role of the nervous system in the pathogenesis of fever, shock and oxygen deficiency. Finally, the important research done on reactivity and narcosis by V. S. Galkin, who untimely death we mourn, and his colleagues, I. I. Fedorov, A. G. Bukhtiarov and others, must be mentioned.

A. A. Bogomolets occupies an outstanding place in the development of Soviet pathophysiology, as do his colleagues and students (N. N. Sirotinin, N. B. Medvedeva, N. N. Gorev, R. E. Kavetsky, R. E. Perelman, Ya. G. Uzhansky and others). A scientist of great scope, he worked out a wide circle of problems – immunity, anaphylaxis, the pathogenesis of shock, questions of constitution, endocrinology, the problem of aging, the problem of blood transfusion and many others. The theory he introduced regarding the role of the active mesenchyme in the realization of a series of physiologic body functions was further developed by his students and colleagues.

The problem of physical reactivity, research on which was based on the studies of I. I. Mechnikov on immunological reactivity, also occupies an extremely important place in the works of Soviet pathophysiologists. This line of research was developed in the work of A. A. Bogomolets and his school (N. N. Sirotinin, I. M. Neiman, R. E. Kavetsky, N. B Medvedeva, R. E. Perelman and others). Important in this context were the studies of G. P. Sakharov, who was one of the first to establish several properties of allergy manifestation (Arthus-Sakharov phenomenon). The influence of the nervous system on the body and the ways in which the body manifests reactivity in a series of pathologic processes were studied by A. D. Speransky and his colleagues, A. D. Ado, D. E. Alpern, V. V. Voronin, A. N. Gordienko, S. M. Pavlenko, Ya. M. Britvan, N. N. Zaiko and others.

The idea introduced by Soviet pathophysiologists concerning the evolution of the body's defense mechanisms is of great importance (A. A. Bogomolets, N. N. Sirotinin and others). This evolutionary approach made possible a better understanding of how certain of the body's defense functions develop.

Finally, we wish to mention the important part played by A. A. Bogomolets in the development of endocrinology in our country.

The problem of functional compensation is closely connected to the problem of reactivity. Investigation of this problem has also been conducted from the point of view that the nervous system plays the leading role. More and more researchers are studying this problem, examining the mechanism and character of compensation in different disturbances (infection and immunity – A. D. Ado, G. V. Peshkovsky; fever – P. N. Veselkin; injured nervous system – E. A. Asratyan, S. Frankshtein; respiratory disturbance – Ya. M. Britvan; loss of blood – R. A. Dymshits, R. E. Kavetsky, D. I. Goldberg and others). Important places in the work of our pathophysiologists are also allotted the study of inflammation (A. A. Bogomolets, D. E. Alpern, A. D. Ado, V. V. Voronin, T. A. Piontkovsky, I. M. Neiman, I. A. Oivin and others) and that of malignant tumors (A. D. Timofeevsky, R. E. Kavetsky and others). Much research investigating the effect of specific factors (electric current, radiant energy) on the body has been conducted by G. L. Frenkel, P. D. Gorizontov and others. Extremely important data on the pathophysiology of the reflex interrelations of the internal organs have been amassed by S. S. Poltyrev and his colleagues.

Soviet pathophysiologists have done work of great practical significance on the problems of hypnoxia and traumatic shock. These problems, as well as blood transfusion methods, were studied by I. R. Petrov, N. N. Sirotinin, N. A. Fedotov, M. E. Marshak, R. E. Kavetsky, A. N. Gordienko, Ya. G. Uzhansky, G. A. Ionkin and others.

The works of G. P. Sakharov, S. S. Khalatov, S. M. Leites, A. M. Charn, S. G. Genes, N. B. Medvedeva and others have done much to develop research on the pathology of metabolism and endocrine regulations.

The groundwork for solving the problem of physical resuscitation has been laid by works of Russian physiologists and pathophysiologists (A. A. Kulyabko, F. A. Andreev). This division of pathophysiology has grown particularly during the years of Soviet rule. V. A. Negovsky and his colleagues studied the basic rules for the removal and restoration of the body's vital functions and created a complex method for treating patients in a critical condition which proved to be of great practical use.

The study of the problems of external pathology is an essential stage in the development of pathologic physiology (M. N. Khanin, O. S. Glozman and others).

The pathogenesis of radiation sickness has been extensively studied by pathophysiologists (P. D. Gorizontov, I. A. Piontkovsky and others). The part played by the nervous system and by the toxic factor has been investigated, and the change in the hematopoietic system and metabolism has been studied as well as the role of the endocrine glands in radiation sickness. Much new data has been already obtained which make it possible to solve certain questions regarding the pathogenesis of this disease. The problems of radiation sickness are attracting more and more attention from pathophysiologists, whose efforts are concentrated in two directions – on one hand, toward the development of the prophylactics and experimental therapy for radiation diseases, and, on the other hand, toward the explanation of the mechanical basis for the therapeutic effect of the radiation factor in a series of pathologic processes.

In 1950, the All-Union Society of Pathophysiologists was organized, uniting the many divisions and departments in different corners of our country. During the forty years of Soviet rule, the number of pathologic physiology departments in the medical institutes has increased considerably. Recently, thanks to the help and support of Soviet Union pathophysiologists, pathologic physiology departments have been included in a series of medical institutes in the countries of the People's Democracy: China, Czechoslovakia, Albania, German Democratic Republic, Bulgaria, Viet Nam and others.

During the years of Soviet power, a new division of pathophysiology, experimental oncology has been developed and instituted as an independent branch of science. Now this new branch has become a border zone in science, attracting the attention of immunologists and biologists as well as that of pathologists.

As early as 1924, A. A. Bogomolets, one of the most outstanding scientists of our country, introduced the theory that malignant tumors cannot develop in a body when that body's active mesenchyme retains its normal resistance. The function of the active mesenchyma is reduced by age, metabolic disturbances, endogenic and exogenic intoxications and incretion disorders.

A new and original trend in experimental oncology was developed in A. D. Speransky's collective (S. I. Lebedinskaya, A. A. Solovyev and others). This trend is genetically connected with the observations of M. K. Petrova, who experimented with the effect of animal neurotization on malignant growth and shows the importance of the nervous system to tumorous growth and metastasis, thereby connecting this "uncontrollable" growth also with the theory of neurism.

R. E. Kavetsky, his students and colleagues have also recently accumulated much data indicating the role of central nervous system disturbances in the pathogenesis of malignant tumors.

The works of N. N. Petrov, the oldest oncologist of our country, made a real contribution to the development of Soviet oncology. His polyetiologic theory of malignant tumor development has found many advocates both here in the Soviet Union and abroad. This theory contained much that was valuable and useful to the problems concerned with explaining the nature of cancerous diseases and was also helpful in the prevention of the so-called occupation cancers.

A very important step was the work of L. F. Larionov, which is being intensively developed. His trophic theory of malignant tumor development is based on the idea that malignant changes can be considered as an adaptive metamorphosis phenomenon of the tissues of higher organisms. Scientists like A. D. Timofeevsky, L. A. Zilber, L. M. Shabad and their students have also made great contributions to research on the etiology of malignant tumors.

An outstanding contribution was made by A. D. Timofeevsky in his works on prolonged cultivation of human tumors which indicated the possibility of the differentiation of malignant cells in a tissue culture. At the same time, A. D. Timofeevsky introduced many new facts germane to the problems of malignant tumor etiology. With the help of the tissue culture and the electron microscope methods, the very important fact that virus-like corpuscles are present in cultures of certain human tissues was established in his laboratory.

L. A. Zilber has done much work on the problems concerning the viral etiology of tumors. He showed that, in certain tumors – Rous's chicken sarcoma and Shope's rabbit papilloma, for example – there are two specific antigens, the tissue and the virus antigens, which are not present in the tissues of healthy bodies. He and his colleagues conducted systematic studies in order to isolate the virus source in other tumors. These data could be very important to the resolution of cancer immunology problems.

L. M. Shabad made a significant contribution to Soviet oncology. He showed that in certain organs and tissues, there are endogenic blastomogenic substances which can cause a tumor to form when introduced into an animal's body. He also developed the idea of a malignancy factor which is detachable from the tissues. Research on carcinogenic substances has been very helpful to cancer prophylactics, since it has made it possible to determine and remove carcinogenic agents from the environment in time, especially the chemical carcinogenic substances.

Lately, Soviet scientists have begun to develop theoretically and experimentally a new branch of the medical-biological division of medicine. N. N. Zhukov-Verezhnikov, I. N. Maisky, P. N. Kosyakov and V. S. Gostev have made significant contributions to this branch. Anti-cancer serums which react in immunological reactions only with the cancer antigen and not with any other antigen have been obtained.

Recently, research in experimental vaccination against malignant tumors has been developed.

Certain new anti-tumor preparations compounded by Soviet scientists (dopan, sarcolysin, *certain antibiotics, etc.) have had notable clinical success.

Observations made on patients treated with dopan make it possible to consider the recommendation of this product for introduction into practical medicine as a treatment for lymphogranulomatosis and the leukoses. Sarcolysin is most effective in the treatment of such tumors as seminomas.

In spite of these achievements, however, the problem of how to treat cancer patients is still far from being decisively solved. Although progress has been made in research on the nature of malignant tumors, why

* Russian trade name.

they occur and how they may be recognized. The treatment of tumors is still one of the main problems of medical science.

In connection with the study of malignant growth immunology, a new medical-biological department has been formed and developed – the immunology of normal and pathologic growth of cells and tissues (non-infectious immunology).

The research done by the main divisions of this department has recently been concentrated in the USSR Academy of Medical Sciences Institute of Experimental Biology. To this institute are referred: embryogenic immunology, research on the immunological mechanisms of tissue incompatibility in transplantations and combinations and the above-mentioned immunology of malignant growth (N. N. Zhukov-Verezhnikov, I. N. Maisky, P. N. Kosyakov, V. S. Gostev, O. E. Vyazov and others). In the course of this research, the presence of immunological interrelationships during the development of both normal and pathologic cells was established to be the general rule. These interrelationships distinguish certain aspects in the development mechanisms of normal cells and underly a series of pathologic phenomena. At the same time, other branches of the new department have been organized to study, for example, the immunology of radiation sickness, the immunology of blood groups and types, etc. Recently, noninfectious immunology has become an important division of not only pathology but experimental biology as well.

In pre-revolutionary Russia, animal biochemistry as a more or less developed science did not exist. There were few scientific workers in biochemistry, although there were some laboratories in Leningrad, Moscow and Kharkov which worked with problems of animal biochemistry and were headed by outstanding scientists (B. I. Slovtsov, S. S. Salazkin and E. S. London in Leningrad, V. S. Gulevich in Moscow and A. V. Palladin in Kharkov). During the first years after the revolution, the rate of biochemistry development in the USSR was mainly determined by the speed with which biochemists could be trained. Active in this task were the laboratory of V. S. Gulevich, part of the Moscow University Department of Medical Science, the Biochemical Institute of Narkomzdrav, organized soon after the revolution under the direction of the outstanding biochemist, Academician A. N. Bakh, and the Ukrainian Biochemical Institute in Kharkov, headed by Academician A. V. Palladin. These laboratories and institutes became the main biochemical schools in the Soviet Union, which produced the most prominent biochemists whose works commanded universal recognition. Somewhat later, a similar school of young cadres was established at the Biochemical Department of the All-Union Institute of Experimental Medicine in Moscow, which was subsequently reorganized to become the USSR Academy of Medical Sciences Institute of Biological and Medical Chemistry. Thanks to these scientific institutions, biochemistry in the USSR began to rapidly overtake the leading countries of Europe and America in this field in respect to both the scope and significance of research and gained a prominent place in world biochemistry.

Early in the thirties, the aerobic resynthesis process connected with cellular respiration of adenosine triphosphoric acid (oxidizing phosphorylation) was discovered by Academician V. A. Engelgardt – a most important discovery. Subsequently, the problem of oxidizing phosphorylation became one of the central problems of biochemistry; V. A Belitser particularly obtained very important data on this problem. At the end of the thirties, V. A. Engelgardt and M. N. Lyubima made another outstanding discovery. They established that the protein myosin, which is responsible for muscle contraction, has the same properties as the enzyme adenosine-triphosphatase (ATP), i.e., that myosin catalyzes the reaction liberating the energy-rich phosphate from ATP, which reaction represents the proximate source of muscular contraction energy. The immense significance of this fact to the physiology of muscular contraction became particularly clear when, after this (V. A. Engelgardt and colleagues), it was established that myosin does not only break down ATP but is itself affected, changing its mechanical and physicochemical properties upon its combination with ATP.

These works initiated a new field of research which was called "the mechanochemistry of the muscle."

D. L. Ferdman established the presence of a specific enzyme, closely linked with myosin, which possessed the ability to deaminize the adenylic acid which forms when ATP is split up; A. M. Utevsky did work of great merit when he solved the important problem of adrenalin and sympathin metabolism in the body.

I. I. Ivanov also conducted research of great importance on the biochemistry of the contractions of various organs.

Of exceptional importance to biochemical research on nitrogen metabolism was A. E. Braunshtein's discovery in 1937 of the reaminization process of amino acids and his subsequently formulated theory concerning

the integration of the intermediate nitrogen metabolism in the animal body. The importance of the reaminization process discovery to the development of the most important problems of nitrogen metabolism becomes more evident each year.

The research of Academician A. V. Palladin and his colleagues deserves an important place in the biochemistry of the central nervous system. The results he obtained from his many years of research studying the chemical composition of morphologically and functionally different parts of the brain and the rules he discovered in the processes of substance metabolism attending the stimulation and inhibition of nervous system functions are the basis for all contemporary central nervous system biochemistry. An important contribution was made by the studies on the biochemistry of the nervous system which were carried out in laboratories under the direction of G. E. Vladimirov. These works explained several pressing questions concerning the metabolism of nucleinic acids and other compounds in the brain and in other sections of the central nervous system.

The research in the field of muscular extractive substances which made V. S. Gulevich world famous continued to be effectively developed in V. S. Gulevich's laboratory even after the revolution. Here we wish to mention N. F. Tolkachevsky's discovery of anserine, concurrent with but independent of Akkerman's discovery of it. After the death of Academician V. S. Gulevich, the leading place in world literature on muscle extractives was taken by the works of S. E. Severin, who conducted extensive research on carnosine and anserine metabolism in the muscles and the role of these compounds in muscle function.

Research on the chemistry and biochemistry of protein began to be rapidly developed in the Soviet Union during the thirties. The works of D. L. Talmud, S. E. Bresler and A. G. Pasynsky on the chemistry and physicochemistry of protein are widely known. V. N. Orekhovich discovered a new type of connective tissue proteins which was called "procollagens." These studies attracted the prolonged attention of many outstanding scientists in different countries, and recently, the question of the role and function of the procollagens has become the subject of lively discussion. Research on protein metabolism with different physiologic and pathologic conditions of the body has developed very rapidly. S. R. Mardashev and his colleagues conducted extensive and profound research on the metabolism of aspartic acid, asparagine and other amides of amino acids in bacteria and in animal bodies; new methods of determining these compounds were developed and their role in the processes of nitrogen metabolism in the body explained.

In works conducted by S. Ya. Kaplansky and his colleagues investigating the mechanism of metabolic disturbance in protein deficiency, much light was thrown on the pathogenesis of disorders due to different forms of exogenic and endogenic protein deficiencies and the changes in the metabolism of the amino acids and other compounds underlying these pathologic phenomena were explained. Extremely important work has also been done explaining the changes in the interrelations between blood serum and liver proteins in various diseases.

B. I. Zbarsky and A. I. Parshin, Yu. M. Gefter conducted extensive research on protein and amino acid metabolism in various tissues, which helped to explain the mechanism of metabolic disturbances in various diseases, especially in malignant neoplasms.

Recently, research in the chemistry and biochemistry of various hormones, a branch of research which has been relatively neglected in the Soviet Union has made progress, especially where the hormones of the suprarenal cortex are concerned. In this context, we must note the studies conducted by N. A. Yudaev in the USSR Academy of Medical Sciences Institute of Biological and Medical Chemistry, which have already given results valuable to the explanation of the synthesis mechanism of various corticosteroids in animal bodies.

Finally, we must mention the important work in the field of comparative biochemistry directed by E. M. Kreps.

From the data presented, it is clear that animal biochemistry has developed rapidly in the Soviet Union since the revolution and that it has been of much help in solving various important questions of physiology, pathology and medicine.

In this short outline, it is difficult to give a sufficiently comprehensive description of how Soviet pharmacology has developed over this forty-year period. In order to give a general idea of the trend of this development, we shall limit outselves to a description of the main problems which confronted pharmacologists during this period, which largely determined the course of pharmacological development as well as the present state of pharmacology.

The development of Soviet pharmacology is closely linked with the name of one of its founders, N. P. Kravkov. Much attention was given in his works to the problems of research methods, especially methods of isolating organs. These studies were not an end in themselves; they were only a necessary stage in the process of solving the main problems. Based on these studies, a series of interesting works were subsequently completed which examined an isolated heart, isolated endocrine glands, coronary and other vessels and also treated the smooth musculature (intestines, uterus) and other objects. The data obtained from these studies established a series of important rules for the change in the reactivity of various animal (and human) organs under different experimental conditions. N. V. Kravkov's work was further developed in research conducted by V.V. Savich, G. L. Shkavera, M. I. Gramitsky, A. I. Kuznetsov, S. V. Anichkov, V. V. Zakusov, B. V. Sentyurin, S. Ya. Arbyzov and others.

During the past forty years, special attention has been given to the development of the pharmacology of the nervous system (V. V. Zakusov, S. V. Anichkov and colleagues). For this purpose, it was found necessary to investigate the reactivity of separate sections of the reflex arc in different pathologic conditions and to study the pharmacological effect on the disease process when the pathologic impulses in various sections of the reflex arc are weakened or temporarily removed; gangliolytics, spasmolytics, curare-like substances, neuroplegic substances, anesthetizing substances and others have been investigated in this connection (S. V. Anichkov, V. V. Zakusov, M. Ya. Mikhelson, V. A. Sanotsky).

Still another field of pharmacological research must be mentioned – one which I. P. Pavlov considered very important. This is experimental tnerapy. This field has been successfully and productively developed in different directions (A. I. Cherkes, V. A. Sanotsky, P. V. Rodionov, N. V. Lazarev, S. Ya. Arbuzov and others).

As well as the study of different medicinal preparations in conditions of "pathologic pharmacology," research on the combined effect of such medicinal preparations is very important from both the theoretical and practical points of view. Much interesting and useful work has been done in this connection (Ya. Kh. Nolle, K. D. Sargin, V. V. Vasilyevich, M. P. Nikolaev, N. V. Lazarev and others).

Questions of age pharmacology have also been investigated in the works of Soviet scientists (I. S. Tsitovich, I. A. Arshavsky and others).

Another branch of pharmacology, experimental chemotherapy, has made great progress. Many preparations for the treatment of various infectious diseases have been obtained and examined (P. F. Gauze, Z. V. Ermoleva, Sh. D. Moshkovsky, G. N. Pershin, Kh. Kh. Planelyes and others).

Toxicology has developed parallel with pharmacology. In works by several Moscow, Kiev, Leningrad and Kharkov scientists, extremely important general rules for the toxic effect of chemical substances have been partially established, and a considerable number of individual examples have been studied in the plan (A. A. Likhachev, S. V. Anichkov, A. I. Cherkes, Z. M. Yavich, V. A. Sanotsky, N. V. Lazarev, Yu. V. Drugov, D. Ts. Zakutinsky, N. S. Pravdin and many others).

The results effected by the pharmacological use of medicinal substances and poisons are undoubtedly influenced to a high degree by the internal conditions of the body. Biochemical research has long been conducted in order to develop this aspect of the question, as well as to discover the way in which the effect of such medicinal substances on the body is realized; biophysical and electrophysiological research has recently also been conducted for this purpose. Many interesting and extremely important findings were obtained from these studies. This research is still being developed and is gradually increasing in range (V. V. Savich, V. I. Skortsov, A. I. Cherkes, V. M. Karasik, G. V. Vasilyeva, G. N. Pershin, G. A. Ponomarev, M. A. Angarskaya, K. A. Shmelev and others).

We had no native pharmaceutical industry before the revolution; the large majority of medicinal preparations came from neighboring countries. We now, however, have several pharmaceutical factories and, more important, a few chemicopharmaceutical institutes, which are systematically working out problems of pharmaceutical chemistry – the Ordzhonikidze All-Union Institute for Scientific Research in Chemicopharmaceutics (VNIKhFI) in Moscow, its affiliate in Sverdlovsk and similar institutes in Kharkov and Tbilisi. Moreover, a great deal of interesting work is being done in other institutions on the synthesis of medicinal substances – in the USSR Academy of Sciences, in the Armenian SSR Academy of Sciences, etc. Much work has been done on the utilization of medicinal substances extracted from plants.

To show how effectively work has developed in this field, we can only mention that the Pharmacological Scientific Committee of the USSR Ministry of Public Health Medical Council has authorized the issue of more than 600 preparations in the last five years alone.

To accomplish all this, great and profound work has been required on the chemical synthesis of various types of chemical compounds (R. M. Mndzhoyan, V. R. Khromov, N. S. Kochetkov and others).

Besides the mass of experimental and organizational work which has been done during the years of Soviet power, an immense amount of literary work has been accomplished. Textbooks, practical handbooks, reference books, every kind of outline, manuals, etc. have been written.

Therefore, our native pharmacology has been developed during this period and has made great progress.

There are problems before experimental pharmacology, the solution of which is of both practical and theoretical importance. The primary mechanisms of pharmacological action, the mechanisms of pharmacological effect on the nervous regulation of functions, the mechanisms of changes in body reactivity induced by pharmacological substances and many other problems require more extensive investigation.

At the same time, work must be intensified on the search for and the examination of new, more effective medicinal substances from different series of chemical compounds for use in the treatment of angina pectoris, radiation diseases, malignant neoplasms, infectious diseases and other affections.

It would be extremely difficult to describe all the achievements made during the past forty years in experimental biology. Since experimental biology is a most extensive branch of science with numerous divisions, we will consider in this article only the part of experimental biology which can be termed medical experimental biology or which is definitely related to the latter.

We shall not, therefore, discuss the progress of the new "michurine biology" or what has been done to develop the evolutionary theory.

The answers to all the main questions concerning the other branches of experimental biology can be found on the pages of the special biological, botanical and zoological journals.

Soviet biology, like other branches of Soviet science, was developed from the famous traditions laid down in the pre-revolutionary period. The ideas of I. I. Mechnikov, the brothers Kovalevsky, K. A. Timiryazev and K. Ber were not only studied but developed by Soviet biologists. The materialistic traditions of Russian biology have matured and developed in the post-October period. This was aided by the organization of many specialized scientific research institutes during the early years of Soviet power. In 1917, the Institute of Experimental Biology was organized, later renamed the Institute of Cytology, Histology and Embryology. The Institute of Experimental Morphogenesis was active from 1930 to 1941 and in 1945 the USSR Academy of Medical Sciences Institute of Experimental Biology was organized. Besides this, there were several laboratories conducting research in experimental biology: the AN SSSR Laboratory of Experimental Zoology and Morphology, the Moscow Zoological Gardens Laboratory of Experimental Biology, several laboratories in the AMN SSSR Institute of Experimental Medicine, etc.

One of the first to organize research in experimental biology was N. K. Koltsov, who founded the Narkomzdrav Institute of Experimental Biology. In this Institute, physicochemical and embryologic studies were conducted. N. K. Koltsov himself is responsible for important research concerned with how the delicate structure of cells is affected by the chemism of their environment. Many prominent researchers, who were subsequently to head new directions in science, were closely associated with N. K. Koltsov (M. M. Zavadovsky, A. S. Serebrovsky, S. N. Skadovsky and others).

M. M. Zavadovsky, who did important work on the role of the internal secretion glands in the formative processes, had a real influence on the development of experimental biology in the USSR. M. M. Zavadovsky organized the Laboratory of Experimental Biology at the Moscow Zoological Gardens and the Chair of Development Dynamics at the Moscow State University, as well as teaching a course in experimental biology at the university for many years. He had numerous students and colleagues (L. Ya. Blyakher, M. A. Vorontsova, N. A. Ilyin, B. A. Kudryashov, I. A. Eskin, V. F. Larionov and others).

Thanks to their works and to the works of B. M. Zavadovsky and his co-workers, great progress was made in the experimental analysis of the postembryonal development processes, including metamorphasis and moulting.

Experimental embryology began to develop rapidly, in the thirties especially, and this rapid development was mainly due to the works of D. P. Filatov and his many students (V. V. Popov, T. A. Detlaf, G. V. Lopashov and others). D. P. Filatov started the comparative morphological research on developmental mechanics, which was closely associated with the evolutionary theory. A series of works on experimental embryology were conducted under the direction of I. I. Shmalgauzen (B. I. Barinsky, N. I. Dragomirov and others) and also by G. A. Shmidt, L. V. Polezhaev, P. S. Chanturishvili, Z. S. Katsnelson and others. The attention of embryologists has recently been drawn to the development of fish, with emphasis on the early stages of development, the fertilization process (T. A. Detlaf, A. S. Ginzburg) and the effect of hormonal factors on fish development (N. L. Gerbilsky and his co-workers). At the same time, extensive investigation of regeneration was begun. This research was conducted in the Laboratory of Experimental Zoology and Morphology, directed by N. V. Nasonov (P. G. Svetlov, L. N. Zhinkin and others), in the laboratories of the Institute of Experimental Morphogenesis and at the Department of General Biology of the Second Moscow State Medical Institute (L. Ya. Blyakher, M. A. Vorontsova, L. D. Liozner, M. I. Efimov, L. V. Polezhaev and others), in the K. A. Timiryazev Biological Institute (B. P. Tokin) and also at Kharkov University under the direction of E. E. Umansky.

At the end of this forty-year period, Soviet researchers had introduced new ideas on regeneration, in which the principles of the michurine doctrine and the opinions of I. P. Pavlov were used, which considerably advanced this field of research, presently being intensively developed (M. A. Vorontsova, A. N. Studitsky and their colleagues, L. V. Polezhaev).

Much work has been done on the problem of transplantation, analyzing the results of transplanting the internal secretion glands. Certain divisions of the science of transplantation, connected with restorative (conservative) surgery are being successfully developed (transplanted vessels, bones, skin grafts, etc.). A method of corneal transplantation was developed in the USSR (V. P. Filatov). The question of nerve regeneration has been extensively studied (B. S. Doinikov, L. A. Falin, M. L. Borovsky).

The works of A. G. Gurvich, who with his colleagues (L. D. Gurvich, G. M. Frank, S. Ya. Zalkind, A. A. Gurvich and others) worked out the problem of mitogenetic radiation, constitute an unusual aspect of histology and cytology.

The mitotic division of cells is still being intensively studied (S. Ya. Zalkind, G. S. Strelin, I. A. Alov, V. N. Dobrokhotov and others).

Much discussion has arisen around O. B. Lepeshinskaya's idea that cells develop from noncellular living matter. This discussion helped to clarify debatable opinions and to accumulate new data. Since 1948, research in the plan of the michurine doctrine has been developed; there have been experiments tracing acquired characteristics, experiments with vegetative hybridization, tracing the influence of mixed sperm on succeeding generations, etc. for the most part concentrated in the A. N. Severtsov Institute of Animal Morphology, the AMN SSSR Institute of Experimental Medicine and the embryology departments of the universities. Considerable progress has also been made in the histological branch of biology.

After the Great October Socialist Revolution, histology was transformed from a largely static and descriptive science into a dynamic science. This is shown by the fact that evolutionary histology was initiated and developed, and cells and tissues were studied with a view to their functional conditions and to the functional significance of different structures. Histology connects with physiology and biochemistry and uses histochemical methods. The basic problem with which Soviet histologists are occupied is the phylogenic and ontogenic development of cells and tissues and how the cells and tissues are changed by different conditions of body subsistence. In connection with this functional aspect of histology the experimental method is widely used and histology has become a primarily experimental science.

The appearance of many important theoretical works and monographs is characteristic of Soviet science, of morphology especially, as it shows both the high theoretical level of science in the USSR and the extraordinarily favorable conditions for scientific development which exist in this country. In this context, we wish to mention the "Essays on Evolutionary Histology" by A. A. Zavarzin and the "General Biological and Experimental Principles of Histology" by N. G. Khlopin, both of which outline systems of evolutionary histology but from different positions and by different research methods. The book of D. N. Nasonov and V. Ya. Aleksandrov, "The Reaction of Live Matter to External Influences (Denaturation Theory of Damage and Stimulation)," belongs

in the same group of outstanding theoretical generalizations in cytophysiological research. The conception of paranecrosis presented in this work, describing the definite physicochemical and morphological properties of live matter, coincides with N. E. Vvedensky's physiological conception of parabiosis.

Important articles have been written by Yu. M. Lazovsky and M. A. Baron, describing results obtained from research on the functional histology of the stomach and of the serous and other membranes.

The work which has been done on the plasticity of histological structures (S. I. Shchelkunov and colleagues) is a very important branch of histological research.

Important research using the tissue culture method to explain the ability of the cells of various tissues for morphological transformation has been conducted by N. G. Khlopin and A. D. Timofeevsky.

An original method for studying the properties of cells and tissues in changed conditions of existence was developed by F. M. Lazarenko who proposed that particles of celloidin be mixed with crushed tissues for implantation purposes.

Neurohistological research has been given special attention since the October Revolution; these studies were being conducted at separate chairs even before the revolution (A. S. Dogel, K. A. Arnshtein, D. A. Timofeev).

We should mention the outstanding morpho-physiological studies of A. N. Mislavsky and B. I. Lavrentyev on the autonomic and sensory innervation of internal organs, which include contemporary observations on the peripheral neurons. These studies have been successfully continued in the direction of research on afferent inner innervation by a pupil of B. I. Lavrentyev, E. K. Plechkova, as well as by N. G. Kolosov, B. A. Dolgo-Saburov, N. I. Zazybin and their students. In this research, which explains the sensory innervation of most of the internal organs and of certain tissues, much attention was given to the changes in different sections of the afferent systems (the receptors, fibers, synapses) in different functional conditions, both normal and pathologic.

Research on the morphology of the central nervous system has also developed considerably, especially after the organization of the Brain Institute in Moscow.

The works of this institute's collective (E. P. Kononova, I. N. Filimonov, G. I. Polyakov and others), conducted by various methods under the general direction of S. A. Sarkisov, were completed in the field of cytoarchitectonics by the publication of an atlas showing the architectonics of the human cerebral cortex — a unique work which has received high acclaim from both native and foreign specialists. We must also mention the substantial research published by B. N. Klosovsky on the morphology and physiology of the blood supply and the circulation of the blood to the brain.

Interesting work has been done on the histochemistry of the nervous system by A. L. Shabadash, who studied the histochemistry of the carbohydrate metabolism in order to examine the biological organization of the nervous system and also by V. V. Portugalov, who used the histochemical method to study the histophysiology of nerve endings.

Numerous works on the physiology of interoceptors, conducted by Soviet physiologists (K. M. Bykov, V. N. Chernigovsky and colleagues and others), have been morphologically based on the morphological research accomplished concerning the afferent innervation of internal organs. Finally, we must mention the basic research on the anatomy of the lymphatic system which was developed by D. A. Zhdanov and the study of indirect blood circulation (V. N. Tonkov and colleagues, B. A. Dolgo-Saburov and colleagues).

Like normal morphology, pathologic morphology has been essentially reorganized during the last forty years. The question of pathogenesis, always important in pathologic morphology has become the predominant interest of pathomorphology during this period. This fact led to the expansion of experimental research in the field of pathomorphology, usually conducted in conjunction with research on material from autopsies and surgical operations in order to maintain the connection between experimental and human pathology. One of the tasks of experimental research is the creation of experimental models of human diseases in animals, with special attention given the pathogenic, as well as the etiologic, relation of the experimentally induced disease to the corresponding human disease. N. N. Anichkov and his collective of colleagues have done much of the work in creating such experimental models.

Several models of autoinfectious diseases of various organs (the bile ducts, the bladder and liver, the respiratory tracts and lungs, the urinary tracts, the appendix) have been developed, which are caused by injuries leading to disturbances in the organ's function, i.e., with no introduction of microbes.

Comparative pathological studies, which have developed considerably during the Soviet era, have also been represented in pathomorphology. The course indicated by the classic works of I. I. Mechnikov has been followed in these studies.

Soviet pathomorphologists have participated in the experimental resolution of the pathologic and medical problems most necessary to the preservation of public health in this country. Here, we should mention the basic research of I. V. Davydovsky, studying the pathologic anatomy of epidemic typhus, and his original monograph on the science of the wound. The facts which have been obtained have hastened the resolution of such problems. Allergy is one such problem and has been the object of much research, the results of which have given information of the morphology of allergic phenomena in various kinds of organs under different conditions (A. I. Abrikosov and colleagues, B. N. Migunov, D. N. Vyropaev and others). On a model of hyperergic inflammation, D. N. Vyropaev demonstrated clearly the significance of the nervous system in the inflammatory reaction.

Great attention has been paid the reticulo-endothelial system and its significance in normal and pathologic conditions to analyzing the inclusion process of colloid substances and suspensions and to blockade of the reticulo-endothelial system (N. N. Anichkov and colleagues, F. F. Sysoev, N. G. Khlopin).

Among the works on inflammation, the important works of V. G. Garshin must be mentioned; they showed the active participation of the epithelium, its eliminative function in inflammation, which fact explained the nature of the so-called atypical epithelial growth. These works also illuminated many other problems in the pathology of epithelial tissue (metaplasia, for example).

Research on infections processes occupied a significant place in experimental pathomorphology: brucellosis (M. B. Ariel), bone tuberculosis (M. E. Mandelshtam and A. N. Chistovich), intestinal infections (A. A. Valdman, L. S. Bibikova), poliomyelitis (N. A. Robinzon and others), neuroviral encephalitides (S. M. Derizhanov), pneumonia (V. D. Tsinzerling and colleagues, B. I. Ugryumov and others), etc. The research conducted by P. P. Dvizhkov and colleagues on labor pathology and that conducted by N. A. Kraevsky and colleagues on radiation sickness also deserves mention.

The pathogenic trend united pathologic morphology with experimental pathology, physiology and biochemistry and led to complex research, using histochemistry, electron microscopy and analytical roentgenography. As an example of such complex research, we cite the works studying connective tissue (A. I. Strukov, G. V. Orlovskaya), which are of great help in explaining the changes of this tissue which occur with rheumatism.

The outline of research which we have presented gives some idea of the great progress and scope achieved by experimental biology and medicine during the past forty years. Soviet science rapidly recovered from the serious damage dealt by the Fascist invasion and was of great help to the country during the years of the Great National War. This progress was made possible through the great popular respect and support enjoyed by Soviet science and because of the constant attention given Soviet scientists by both the Communist Party and the Soviet government.

The continual progress of Soviet science during the past forty years has been due to the fact that Soviet scientists are directed in their research by the most advanced philosophy – the philosophy of dialectic materialism.

The progress which our country has made during these forty years in experimental medicine and biology guarantees an even greater and more vigorous development of science in the future.

PHYSIOLOGY

MOTOR REFLEXES IN PUPS FOLLOWING EXTINCTION OF DISTANT RECEPTORS

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In recent years more and more work has been devoted to the study of conditioned reflexes at various stages of ontogenesis. Investigation of this – among the most important – side of evolution of the higher nervous activity has included many aspects. At least two series of experiments should be mentioned which determine the general theoretical approach to the study of the higher functions of the organism in onto- or phylogenesis. In one series it is the consistent features in the mechanism of the establishment of conditioned reflexes (temporary connections) that are revealed as well as properties of various analysors and interaction of external and internal factors in the animal's behavior. In the other series of experiments the objective method using conditioned reflexes serves as a most important tool in the study of the establishment of vegetative and somatic functions and of those regulatory mechanisms which are mediated by the higher levels of the nervous system. Concrete problems in each series are of interest in themselves, but the results, which inevitably criss-cross and overlap in a variety of ways, contribute to a fuller knowledge of general and specific laws of development of the young and adult stabilized organism.

In our laboratory [2] during a period of over 20 years studies have been carried out probing into the mechanisms of interoceptive conditioned reflexes and internal analysors, their interaction with exteroceptive external analysors and some correlation of these studies to ontogenesis was made [20, 21, 22, 23]. The material and generalizations of facts of this sort proved, in turn, to be one of the essential aspects of elaboration of those problems which are grouped under the name of cortico-visceral physiology and pathology and are being investigated by the scientific team working with K. M. Bykov [9, 10]. Spurred on by the logical development of our discoveries, we have begun, in recent years, detailed studies of morphologic and physiologic structure of the central ends of internal analysors on the assumption that it reflects the general principle of dynamic localization put forward by I. P. Pavlov.

It was particularly in this connection that a new subject for investigation presented itself to us, viz. cortical compensatory mechanism which had attracted the interest of many investigators [6, 7, 8]. We took V. S. Gaikin's [11] and K. S. Abuladze's [1] results as the point of departure; in their work on simultaneous extinction of 3 distant receptors a profound and irreversible depression of all cortical activity had been found. I. P. Pavlov considered these findings to be highly significant and recommended that they be studied from all possible angles [24, 25].

Our studies begun in 1952 [3, 4, 5] and those of other authors [12, 13, 16, 17, 18, 19] showed that in adult animals subjected to gradual extinction of distant receptors conditions arose under which cortical compensatory mechanisms came into play and a relatively active form of adaptation to new conditions of existence took place. The leading role in this process is taken by the motor analysor which steps in as substitute for vision, olfaction, hearing and vestibular reactions. Among the possible factors determining the degree and time elements in establishment of compensatory functions the type of the animal's nervous system and the time interval between extinction of each analysor have been mentioned.

Work along similar lines carried out by our collaborators (A. S. Batuev, V.Yu. Ermolaeva, K. Libentrau, L. N. Savina, I. V. Orlov) has provided comparative data on fish, squirrels, rabbits, cats and monkeys.

B. N. Klosovsky and E. N. Kosmarskaya [14, 15] reported, after the publication of our work, interesting observations on the general behavior of pups deprived of distant receptors.

In the present communication are presented investigations into mechanisms of substitution of the functions of lost receptors in the dog at various ontogenetic stages.

EXPERIMENTAL METHODS

Experiments were performed on 44 pups of different age groups, using the motor-food technique. During the experiment the pup was placed in a chamber with three ply-wood shields on its floor and rubber bulbs underneath them. The bulbs were connected to Marey's tambours. The pup's movements in the chamber were suitably recorded on a kymograph. A pneumatic feeding device was placed at one end of the chamber. In addition to recording the pup's approaches to the feeding device, its respiration was also recorded. A system of positive and inhibitory conditioned exteroceptive and interoceptive reflexes was developed in all the pups; the former were developed in some to the sound of a metronome, in others to touching or pressing of a paw by a rubber cuff, the latter to inflation of a rubber balloon placed in the stomach by way of a fistula tube. Gastric fistulas were made starting at the age of 3 weeks following which conditioned reflexes were developed. Once the background conditioned reflex activity was established the animals were subjected to separate extinction of the visual and olfactory receptors or, depending on the experimental series, to simultaneous extinction of the visual, auditory, vestibular and olfactory receptors.

EXPERIMENTAL RESULTS

During the first few days following enucleation in 12 pups of all age groups disturbances of motor function were seen in all; these were particularly obvious on attempts to walk upstairs: the pups could not ascend the stairs and had to be carried up. However, as early as the 7th to 8th day after enucleation 5-months old pups could ascend the stairs without assistance, whereas 8-months old ones could do so only from the 12th to 14th day after enucleation.

In addition to motor disturbances pups of all ages showed changes in conditioned reflex activity. There was, as a rule, inhibition of positive motor conditioned reflexes, both exteroceptive and interoceptive, and disinhibition of differentiation.

Figure 1 shows a kymogram of an experiment on a pup before and after enucleation. Prior to operation the latent period of the conditioned motor reflex reaction to distension of the stomach was 3 seconds and to the sound of a metronone – 4 seconds.

Destruction of the peripheral end of the visual analysor was carried out when the pup was 6 months old. On the 5th post-operative day the pup failed to react either to the exteroceptive or the interoceptive conditioned stimuli at the beginning of the experiment, and only began to react toward the end of the experiment by approaching the feeding device; the latent period of the motor-food reaction was 18-20 seconds. By the 7th day this pup, and others of the same age group, showed complete restoration of conditioned reflexes, while 8-months old pups showed such restoration only by the 12-14th day.

It was thus demonstrated that substitution of disrupted cortical function following enucleation was the more effective the younger the animal concerned.

When the peripheral end of the visual analysor was removed in 4 pups before their eyes had opened (aged 8-10 days) a lag in the development of motor reactions was observed. These pups began to move about the cage 7-8 days later than control animals. When one of these pups was placed together with seeing pups the former was less mobile, clung to the floor and continually climbed underneath the other pups. Three-week old control pups (seeing) ran up immediately in response to the experimenter's call or noise made by him with the feeding dish, while the experimental ones approached slowly and not at once. It must be mentioned that pups operated at the age of 1-2 months moved about the cage rapidly and ran up to the food as quickly as control (seeing) pups as early as 2nd to 3rd day post-operatively.

Consequently, removal of as yet nonfunctioning visual receptor and the associated abortive termination of development of the central end of the visual analysor produce profound impairment of cortical activity. As can be seen from our experiments, "rearrangement" of analysors is achieved much more smoothly after the whole cortex had matured harmoniously; the process of substituting for visual function occurs more rapidly in pups operated after opening of their eyes than in those operated before this.

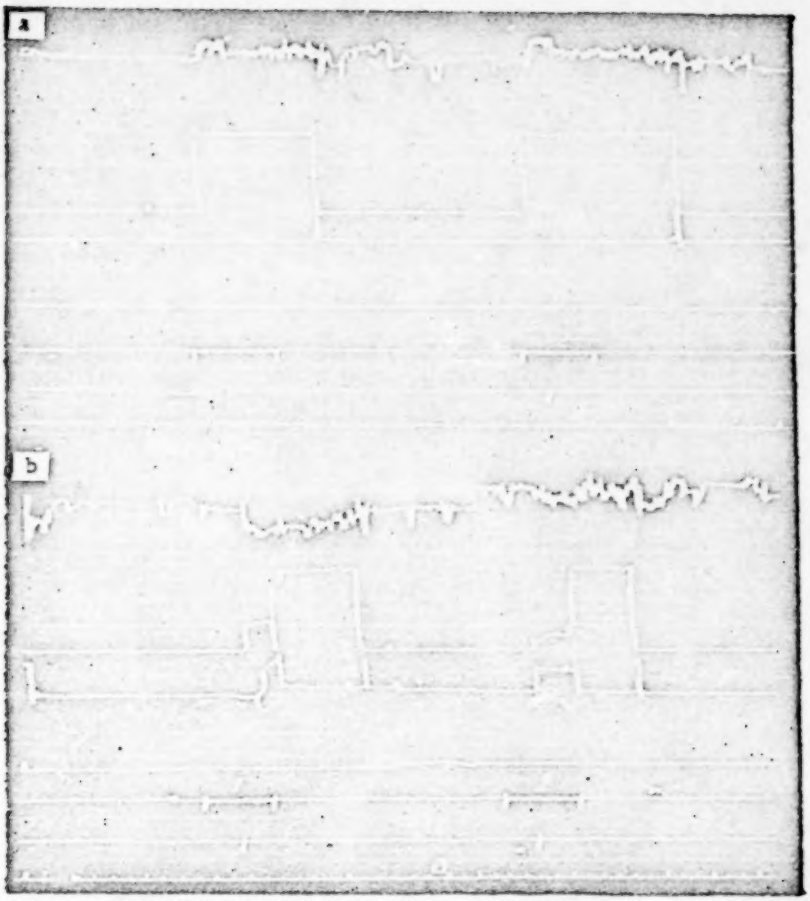

Fig. 1. Conditioned reflexes to distension of the stomach (1) and sound of metronome (2) before enucleation (a) and on the 5th day after enucleation (b) in a pup aged 6 months.
Records from above down: respiration; pedal nearest to feeding device; second pedal further away from feeding device; third pedal still further away from feeding device; conditioned stimulus marker; unconditioned stimulus marker (placing of feeding device); time marker (10 seconds).

The olfactory analysor is known to be well developed in dogs, and its particular role in the postnatal period has been clearly shown in the work of V. A. Troshikhin [26]. Our experiments were performed on 12 pups. Extinction of the peripheral end of the analysor (section of the olfactory nerves) led to inhibition of all positive conditioned reflexes, both extero- and interoceptive, during the first few days after operation; the conditioned reflex to distension of the stomach was inhibited more than others, and among exteroceptive ones the reflex to cutaneous stimulation proved to be the most sensitive one. Apparently the question of "strength"

arises in this connection, since the stimuli mentioned are, undoubtedly, weaker than the auditory one (metronome). The process of compensation following extinction of olfaction takes place the more rapidly the younger the animal, as is the case in enucleation. Thus, for example, conditioned reflexes are restored after 7-8 days post-operatively in pups aged $2^1/_2$ to 3 months, after 12-14 days in pups aged 5 months and after 25-26 days in pups aged 10 months.

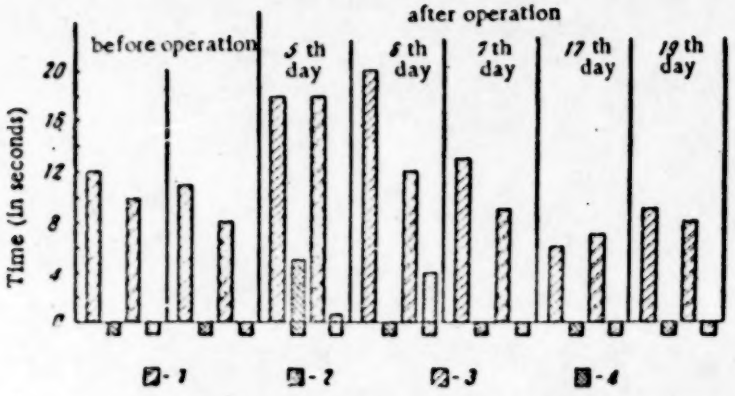

Fig. 2. Average values of latent periods of conditioned reflexes on different days before and after enucleation in a pup aged 5 months. 1) Latent period of exteroceptive conditioned reflex (metronome – 120 strokes per minute); 2) exteroceptive differentiation (metronome – 60 strokes per minute); 3) latent period of interoceptive conditioned reflex to rhythmic distension of stomach; 4) interoceptive differentiation; blocks below zero line – complete differentiation.

Comparative analysis shows, however that extinction of olfaction leads to more pronounced changes in higher nervous activity than that of vision within the same age group of pups. This is illustrated in Fig. 2 (vision) and Fig. 3 (olfaction). The figures represent average values of latent periods for conditioned reflexes in two pups of the same age (5 months).

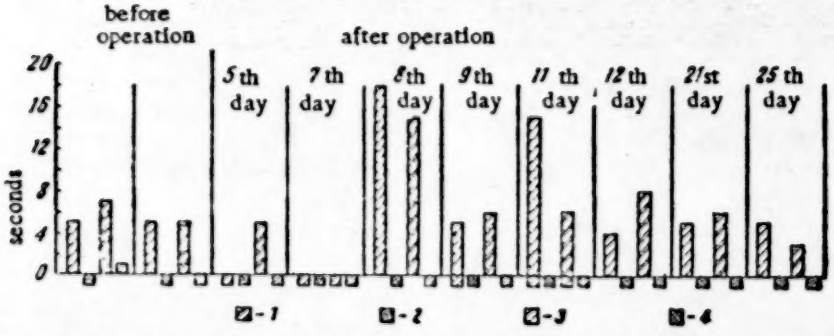

Fig. 3. Average values of latent periods of conditioned reflexes on different days before and after section of olfactory nerves.
Legend the same as in Fig. 2.

Of particular interest are the results of the series of experiments concerned with the study of cortical compensation at early stages of ontogenesis in pups with simultaneous extinction of the peripheral ends of the visual, auditory and olfactory receptors and destruction of the vestibular apparatus. As has already been mentioned, according to data presented by V. S. Galkin and K. S. Abuladze, adult dogs following this procedure went into a prolonged inhibitory state, the ensuing sleep lasting 23 out of every 24 hours. Quite different phenomena became apparent in our experiments on 16 pups operated on at the ages of from 2 to 10 months.

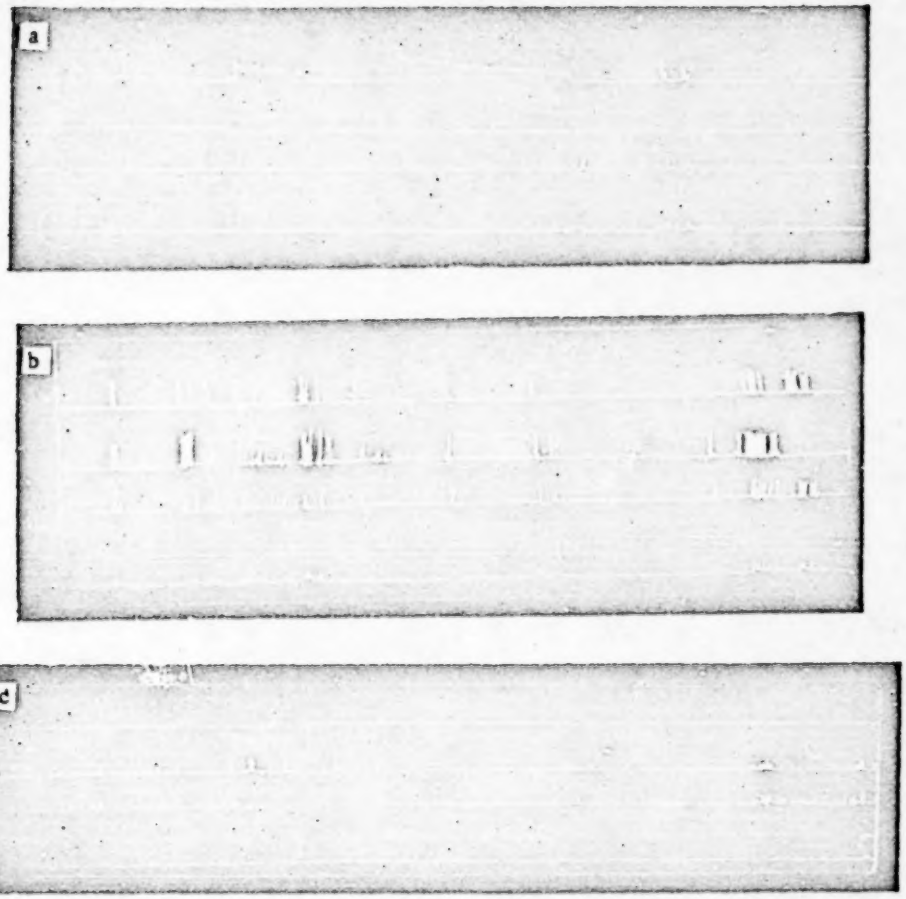

Fig. 4. "Actogram" of the daily activity of 3 pups following operative extinction of 4 receptors (visual, auditory, olfactory and vestibular).
a) 10-months old pup on 18th post-operative day; b) 5-months old pup on 10th post-operative day; c) $2\frac{1}{2}$-months old pup on 2nd post-operative day.

In addition to investigation of their conditioned reflex activity before and after operation, records were taken of the daily activity of the pups. It was found that 10-months old pups subjected to simultaneous deprivation of the 4 receptors mentioned above did not, for a long time after operation, take food spontaneously, did not move about the cage and spent considerable part of the day in an immobile state. On the 12th-15th post-operative day pups of this age group began to move about the cage. Figure 4, a presents records of the daily activity of such a pup on the 18th post-operative day. A different picture emerged in the case of pups operated on at the age of 5 months. They, as a rule, moved about the cage by the 8th-9th post-operative day and by the 10th-11th day their daily activity (Fig. 4, b) differed little from the daily activity of normal (control) pups.

Compensation occurred even sooner at earlier stages of ontogenesis. Pups aged 2-3 months began to walk spontaneously and to maintain prolonged motor activity in the course of a day as early as 2-6 days after simultaneous removal of 4 receptors. Figure 4, c shows the daily motor activity of a 2 ½-months old pup on the 2nd day after operation. The pup moved about the cage spontaneously, took food from a dish and even stood up on its hind legs, resting its front paws on the wall of the cage, without losing its balance.

Pups aged 2-3 months showed as early as the 5th-6th post-operative day well marked orientation reaction, finding the feeding vessel quickly even though they found themselves in the chamber for the first time only after operation. Older animals, subjected to removal of the receptors at the age of 5 months, only began to find the feeding vessel on the 15th-18th post-operative day, while 10-months old pups did not always chose the right direction toward it even as late as the 25th-30th post-operative day.

It follows from the facts cited above that the younger the animal the quicker the establishment of cortical compensatory process (as regards the remaining analysors especially the muscular, and also visceral) even upon simultaneous loss of function of 4 receptors. Of special interest are the dynamics of higher nervous activity mediated by the remaining receptors.*

Do these compensatory phenomena prove to be stable in the course of the animal's further development, becoming ever more complete or, on the contrary, are these phasic phenomena adjusted only to definite ontogenetic periods reflecting high plasticity of young cerebral structure? These and many other questions emerging from the facts and observations described above form the many aspects of our studies.

SUMMARY

Experiments were performed on puppies of various age. The receptors (olfactory, visual, auditory, vestibular) were extinguished in one stage or gradually, one by one. It was established that disturbances which were thus caused are compensated by the cortex cerebri. The younger the animal the better and the quicker the development of this compensatory mechanism.

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ANALYSIS OF MECHANISMS DETERMINING ADAPTATION OF GASTRIC JUICE PROPERTIES TO THE NATURE OF THE FOOD

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As has been previously shown [5], the secretory process in the normal organism occurs in such a way that the gastric juice secreted is best suited to the digestion of that sort of food which is at that time being subjected to digestion. Following ingestion of meat the gastric juice collected from a Pavlov pouch exerts a more pronounced action on proteins of animal origin than on proteins of vegetable origin. The reverse effect is seen upon ingestion of bread. Such fine adaptation is, apparently, aimed at ensuring the most effective treatment of the food in the stomach with the most economic use of the glandular apparatus. The intensity of animal protein digestion has been designated as zoolytic activity of the juice and plant protein digestion as phytolytic activity. In many animals this adaptation is sufficiently labile to become apparent 30-60 minutes after the beginning of feeding. This evidently has the reflex nature of initial adaptation, while adaptation of the gastric juice in subsequent hours can be explained in two ways: humoral adaptation mechanisms may be postulated but also long-acting nervous influences may be involved. The present work is devoted to an analysis of the regulatory mechanisms responsible for the adaptation of gastric juice to the type of food ingested.

EXPERIMENTAL METHODS

Chronic experiments were carried out on 2 dogs with esophagotomy and gastric fistula and on 2 dogs with isolated Heidenhain pouch. Experiments with "pseudo-feeding" were performed on animals, which had been on a mixed diet, 16-20 hours after the last feeding. The stomach was washed out with warm water; the digestive power and acidity of "spontaneous" portions of the juice were then determined. The animals were given meat in one lot of experiments and white bread in another lot of experiments. Ten to fifteen minute samples of the juice were examined. Animals with the Heidenhain pouches were on a mixed diet and following 16-18 hour fasting received 200 g meat or 250 g bread. The digestive power of the juice was determined on half-hourly and hourly samples.

The proteolytic properties of gastric juice were determined parallelly on proteins of animal origin (mixture of denatured muscle proteins) and on proteins of plant origin (gluten). To a weighed amount (50 mg) of each of the proteins was added 0.3, 0.5 or 1 ml of gastric juice obtained. Incubation at 35-38° was continued for 20-30 minutes. Enzyme activity was determined in the early experiments by the undissolved residue of protein, in the later ones it was mainly determined by the Anson-Mirsky [6] tyrosine method. Results obtained by the latter method are reported in the present work, although they do not differ qualitatively from those obtained by the former.

EXPERIMENTAL RESULTS

Excitation of the receptor elements of the oral cavity and, to a lesser extent, of the pharynx which occur during eating acts as a direct stimulus for the initial period of gastric secretion [1, 3]. The question arises whether this "ignition" secretion has definite adaptive features with respect to the nature of ingested food or whether it is, as it were, indifferent and adaptation is achieved at some other stage of the process ?

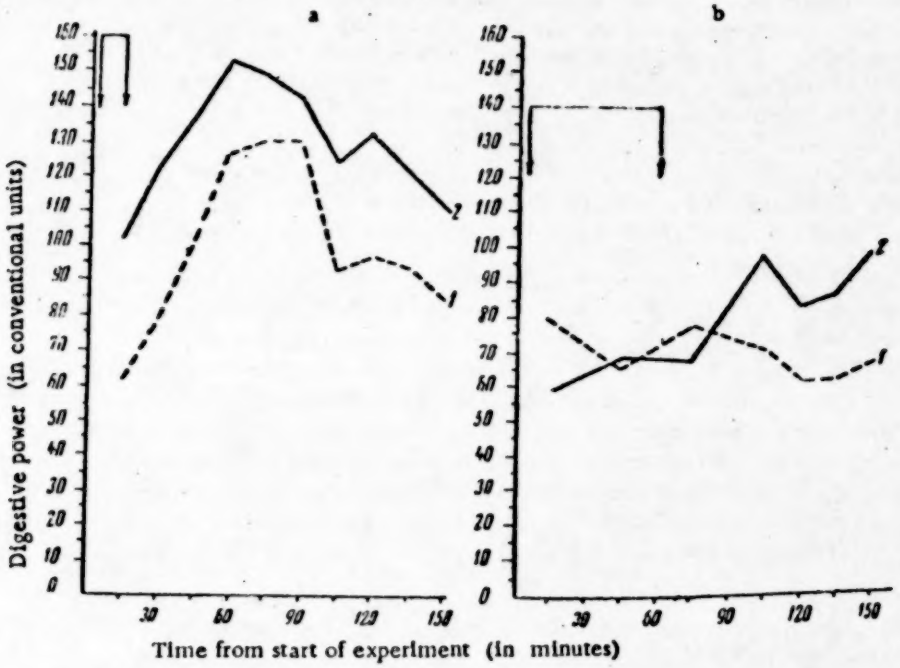

Fig. 1. Breakdown of plant (1) and animal (2) proteins in vitro by gastric juice of dogs upon "pseudo-feeding" with meat (a) and bread (b). Arrows indicate period of feeding.

The first experiments showed that with pseudo-feeding with meat the majority of samples collected during 1-1½ hours (each taken at 10-15 minute intervals) showed predominance of activity with respect to animal proteins. It is suggested that activity of enzyme or secretion toward substrates of animal origin be called zoolytic and toward substrates of plant origin – phytolytic or phytotropic. Thus, even excitation of the oral receptors only by food is sufficient not merely to initiate the secretory process but also for adaptive features to develop. In the final analysis this must mean that the receptor apparatus and central nervous mechanisms possess sufficiently specialized and differentiated properties to ensure reflex adaptation of the quality of gastric juice to the nature of the food.

If the times of feeding are noted carefully the following phenomenon becomes apparent (Fig. 1). Samples of juice collected during feeding and immediate y afterward show definite adaptation to the nature of the food stimulus, whereas subsequently the juice acquires intermediate properties although secretion remains fairly vigorous. This evidently signifies that there is less aftereffect on adaptation than on secretion as a whole, following stimulation.

Figure 1, a shows that when the animal is given meat, adaptation of gastric juice to the food is most marked in its first portions. The ratio of phytolytic activity to zoolytic activity (designated as an index P/Z) at this time is found to be low. On cessation of feeding the index rises until an indifferent level for the given animal is reached (0.7-0.8). Figure 1, b shows that the first samples of gastric juice, coinciding with feeding

of bread, break down plant proteins more readily than animal. On cessation of pseudo-feeding the relative plant protein break-down activity drops while break-down activity for animal proteins rises so that the P/Z index fairly quickly reaches a stable level of 0.7-0.8 (similar to that in the experiment with meat).

Statistical treatment (Fischer-Student) of the data obtained showed the whole of the results to be completely reliable as regards comparison of all samples of "meat" and "bread" juices during periods of pseudo-feeding (P less than 0.001). There is significant difference in the properties of those samples of "bread" or "meat" juices collected during the period of pseudo-feeding on the one hand and during reflex aftereffect on the other.

It has already been noted that indices for juice obtained in response to meat and bread outside the periods of pseudo-feeding are fairly close together. This is confirmed by statistical treatment of all the material showing lack of significance in the differences obtained. The following phenomena occurring upon pseudo-feeding should, therefore, be distinguished. Pseudo-feeding is associated not only with excitation of secretory activity of gastric glands but also with such modifications of the secretory process that the proteolytic activity of the secreted juice becomes adapted to the nature of the food ingested. Soon after cessation of pseudo-feeding, when intensive secretion still continues, the juice loses its qualitative adaptation to a particular type of food to such an extent that it becomes impossible at any rate using our technique to distinguish the juice obtained in response to meat from that obtained in response to bread.

The reflex arising in response to pseudo-feeding is therefore not simply secretory but adaptive secretory. This raises the question whether stimuli which evoke secretion also evoke adaptation or whether the two factors have a different structural basis. The material cited indicates that the two phenomena are distinct since secretory influences are maintained for considerably longer periods than adaptive ones.

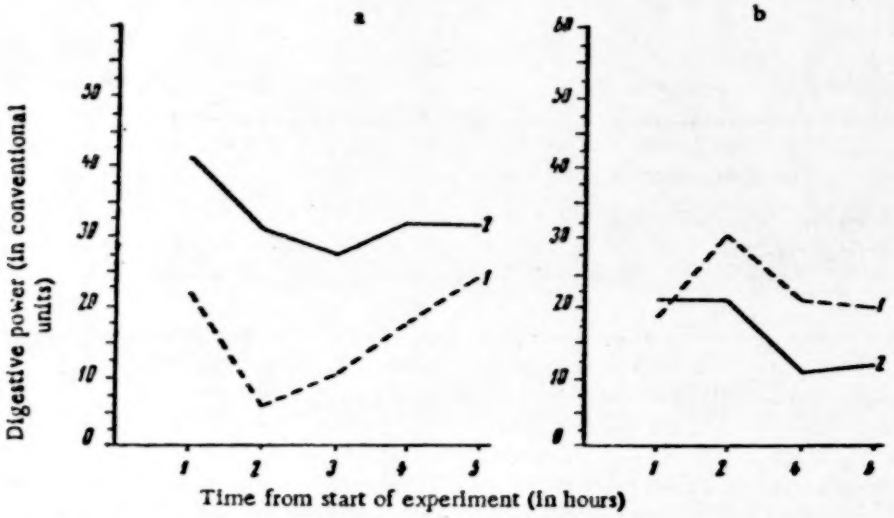

Fig. 2. Breakdown of plant (1) and animal (2) proteins by juice obtained from Heidenhain pouch in response to meat (a) and to bread (b).

Of some interest, from this point of view, are some other observations made by us. In those cases in which the animal consumed the food greedily adaptation of the digestive properties of the juice to the nature of the food was clear and definite, whereas the juice secreted in response either to meat or bread in cases in which the animal ate without desire was very close to indifferent.

It was noted previously that in juice obtained from a Pavlov pouch adaptation changes in the digestive properties of the juice could be detected very quickly; these changes persisted for a fairly long time including

that period which is known as the humoral-chemical phase of secretion. At the same time it was also shown that on pseudo-feeding adaptation occurred fairly rapidly but also disappeared rapidly. This leads naturally to speculation as to how would adaptation processes develop in a stomach completely or almost completely deprived of parasympathetic innervation. In this connection experiments were carried out on dogs with Heidenhain pouches.

As can be seen from Fig. 2, despite considerable changes in the secretory process typical for denervated pouch [2, 4] and appreciable lowering of digestive power as a whole, the adaptive capacity of the secretory apparatus is preserved. Although the amount of secretion is diminished, the ability of the Heidenhain pouch to secrete juice better able to digest meat proteins in response to meat and plant proteins in response to bread is maintained. The P/Z index provides no basis for suggesting that there is any reliable lowering of this ability.

At the same time it is impossible to neglect one detail which sharply distinguishes the gland adaptation process in the Pavlov [5] and the Heidenhain pouches. It has already been pointed out that adaptation of the digestive properties of juice from a Pavlov pouch can be detected in samples collected in the first 30-60 minutes after beginning of feeding; in the case of the Heidenhain pouch the latent period of secretion is 30-40 minutes so that the first determination of the digestive properties was made on samples of juice collected in the 2nd hour. Fairly frequently the ratio of phyto- and zoolytic activities was not commensurate with the nature of the food given (Fig. 2). Adaptation of digestive properties occurred at later periods.

The most noticeable difference in the process of adaptation in the denervated pouch is thus its inertness.

The material so far obtained gives positive evidence of adaptation in the Heidenhain pouch but does not as yet reveal the underlying mechanism. However, it can be asserted even now that adaptation is achieved in one of the following ways. Since denervation which occurs in the process of formation of a Heidenhain pouch is always incomplete it may be supposed that adaptation is preserved so long as parasympathetic innervation is present. It may also be supposed that parasympathetic innervation is practically entirely interrupted during the formation of a Heidenhain pouch. In that case adaptation may be mediated by the remaining sympathetic innervation. Finally, the possibility of the existence of specialized humoral mechanisms responsible for adaptation of gastric juice properties during the second phase of gastric secretion cannot be excluded.

In summarizing all the material presented in this paper it can be stated that reflex mechanisms which determine adaptation of gastric juice properties in the first phase of secretion do exist. Their activity is stimulated by excitation of receptors in the oral cavity upon intake of food. Prolonged maintenance of adaptation of digestive properties to the nature of the food is apparently ensured by a supplementary mechanism which is activated by food when it gains access to the stomach. This mechanism is found in sufficiently "pure" form on examination of juice obtained from a Heidenhain pouch.

SUMMARY

It was demonstrated that adaptive changes of the proteolytic properties of the stomach juice to the quality of food appear not only during the normal course of digestion, but, likewise, in pseudo-feeding. Thus, in pseudo-feeding by meat, there is a prevalence of the zoolytic activity of the gastric juice (the splitting of proteins of animal origin). In pseudo-feeding by bread, phytolytic (splitting of fats of vegetable nature). Adaptation of the proteolytic properties of the gastric juice disappears in 20-30 minutes after pseudo-feeding is discontinued, although the secretion still continues.

Adaptation of the properties of the gastric juice from the Heidenhain pouch is, likewise, pronounced and persists for several hours. However, its development is much slower than in the case of Pavlov pouch.

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* In Russian.

ROUTES OF TRANSMISSION OF CEREBRAL CORTICAL INFLUENCES TO THE THYROID GLAND

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The regulatory action of the central nervous system on the thyroid gland has now been generally accepted.

However, no final answer to the question of the routes by which this influence is transmitted has yet been found.

Some authors consider that impulses sent by the CNS reach the thyroid only by way of the thyrotropic hormone of the hypophysis [9, 10, 11, 12]. Others hold the view that cortical influences can reach the thyroid by direct neural pathways, without pituitary mediation. In a number of cases this view is based, however, on the assumption that the presence of extensive innervation in the thyroid cannot be without significance in the regulation of its secretory activity [1, 4, 7, 8]. Some authors limited their search for pathways for cortical influences to experiments with extirpation of the pituitary [2].

The present investigation is devoted to a study of routes of transmission of cortical influences to the thyroid, using electrodefensive conditioned reflex activity technique.

EXPERIMENTAL METHODS

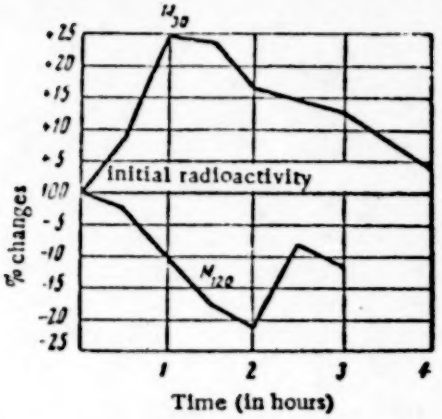
In order to study the influence of the cerebral cortex on thyroid activity conditioned electrocutaneous defensive reflexes to the sound of a metronome – 120 strokes per minute – displaced from the unconditioned stimulus by 20 seconds, were established. Differentiation was developed to 30 strokes of the metronome per minute. Electric current from an induction coil, exceeding the threshold strength by 1-2 cm was used as the unconditioned stimulus.

The functional state of the thyroid gland was determined by estimating its content of radioactive iodine. Radioactivity of the thyroid was measured by a gamma-ray ("AMM-4") counter tube placed in a thin-walled aluminum cover and connected with the apparatus by a flexible screened cable. Such a tube was firmly attached by means of a collar to the neck over the thyroid. Radioactive iodine was introduced as the sodium salt, without carrier, in doses of 2-5 μ cu per dog. Thyroid radioactivity was measured 48 hours after administration of labelled iodine when no more absorption could be detected. Counts were made every hour over a period of 3-4 hours while the animal was in the chamber. Percentage changes were calculated with respect to the initial radioactivity which was taken as 100%.

At the beginning of this work a careful study was made of fluctuations in radioactive iodine levels in the thyroid in response to the dog being placed in the chamber and in the special stand. It was found that the radioactive content of the thyroid remained unchanged under these conditions, the fluctuations in its level being within physiologic limits and not exceeding $\pm$ 10%.

EXPERIMENTAL RESULTS

The use of electric current as unconditioned stimulus enhances secretion of radioactive iodine by the thyroid. As early as one hour following a single stimulation of the paw by electric current there is appreciable decrease in radioactive iodine in the thyroid; maximal drop in radioactivity is observed after 2 hours, the average percentage of change being −20.1 ± 1.3. Only after 3-4 hours a tendency to restoration of radioactive iodine content to the initial value becomes apparent.



Effect of defensive conditioned reflexes on radioactive iodine content of the thyroid gland.

It is known that the electrocutaneous defensive conditioned reflex is usually established very rapidly. Another distinctive feature of this reflex is that if it is reinforced only in those cases when the conditioned reaction is absent the conditioned connection is established extremely quickly and the dog does not go into a state of inhibition [3, 5, 6]. In making use of this feature of the defensive reflex it was possible to stage experiments without reinforcement over prolonged periods of time; this enabled us to observe function of the thyroid subjected to impulses from the cerebral cortex only.

The radioactive iodine content of the thyroid falls under the influence of positive conditioned reflexes. This reaction becomes apparent an hour after placing the animal in the chamber and reaches a maximum at the end of 2 hours. The average percentage of changes equals 21.2 ± 4.6 (see figure). The thyroid reacts in almost the same way to the unconditioned electric stimulus.

Thus both the unconditioned stimulus and the conditioned defensive reflex established on the basis of electric reinforcement are able to stimulate the secretion of products of thyroid activity into the blood to practically the same degree.

The influence of differentiation inhibition on secretion of radioactive iodine by the thyroid was studied in those experiments in which the inhibitory stimulus was used in isolation; this stimulus, similarly to the positive stimulus, was applied up to 10 times in the course of the first hour, with 5-minute intervals. The figure shows that as early as 30 minutes after application of M30 increased radioactive iodine content in the thyroid can be observed; during the subsequent 2 hours the gland's radioactivity remains at a high level and only after 3-4 hours returns to the original level. In those cases in which differentiation was disinhibited thyroid radioactivity fell, i.e., a reaction corresponding to the action of the positive metronome frequency was observed.

These investigations indicate that changes in thyroid activity take place depending on the functional state of the CNS. Excitation of the cerebral cortex is associated with increased secretion of radioactive iodine by the thyroid and, consequently, secretion of products of its activity is increased; when the cortex is in a state of inhibition accumulation of labelled iodine in the thyroid is observed, indicating slowed secretion of products of its activity into the blood.

What are the mechanisms mediating the influence of cerebral cortex on thyroid function?

As already mentioned, a number of authors at present consider that CNS influences on the thyroid are mediated exclusively by the hypophysis. To discover whether the hypophysis was indeed the sole humoral link in the regulation of thyroid secretory activity a series of experiments on hypophysectomized animals was carried out. The results showed that under the influence of defensive conditioned reflexes stimulation of secretion of the products of thyroid activity into the blood occurred in these animals in the same way as prior

to operation; this reaction was, it is true, not as constant as in intact animals since in a number of cases a converse effect was observed, viz. accumulation of radioactive iodine in the thyroid, and in some cases no effect at all was detected. Nonetheless these data demonstrate that changes in the functional state of the cortex can be felt in the activity of the thyroid even in the absence of the hypophysis (Table 1).

TABLE 1

Effect of Electrodefensive Conditioned Reflexes on Radioactive Iodine Content of the Thyroid in Hypophysectomized Animals (Changes are Given in Percentage with Respect to Initial Radioactivity)

Date of experiment	Change in radioactive iodine content of the thyroid time (in minutes)					
	60	90	120	150	180	210
28/V 1956	0	+11	−22	−32	−32	—
29/V	0	+16	+16	—	—	—
30/V	−14	−21	−14	—	—	—
31/V	+9	--	+2	−4	−8	—
4/VI	−12	--	−17	—	−17	—
8/VI	−37	—	−22	—	−15	—
22/XI	+20	+18	+14	+18	+20	—
26/XI	0	—	+9	0	+9	+9
28/XI	+18	+18	+36	+22	—	—
29/XI	−3	--	+2	—	0	+10
3/XII	−20	--	−25	—	−30	—
4/XII	−21	—	−26	—	−16	—
10/XII	+16	—	+8	—	—	—
11/XII	−15	—	−35	—	−15	—
12/XII	+47	--	+24	—	—	—
13/XII	−24	--	−24	—	−24	—

TABLE 2

Effect of Electrodefensive Conditioned Reflexes on Radioactive Iodine Content of the Thyroid After Removal of the Right Adrenal and Transection of the Greater Splanchnic Nerve on the Left (Changes are Given in Percentage with Respect to the Initial Radioactivity)

Date of experiment	Change in radioactive iodine content of the thyroid time (in minutes)						
	60	90	120	150	180	210	240
22/VI 1956	+10	+29	+22	+16	—	—	—
25/VI	—	—	+21	+18	+18	+7	—
26/VI	−22	—	−22	−17	−16	—	—
28/VI	−18	−16	−5	−11	—	—	—
2/VII	+2	+12	+2	+3	−12	0	—
6/VII	−24	−38	−34	—	−28	—	−20
14/VIII	−2	−2	+9	−2	−2	+4	+6
16/VIII	−17	−17	−14	−10	−5	—	—
17/VIII	−17	−14	−12	−11	−9	—	—
29/X	−51	--	−31	—	−5	—	—

For further analysis of the mechanism of transmission of cortical influences to the thyroid an attempt was made to elucidate the role of the adrenals. With this in view, the adrenal gland was removed from one side and the splanchnic nerve was transected on the other. The hypophysis was not removed in these dogs. Experiments were performed 1-3 weeks after operation. Table 2 shows that under the influence of positive conditioned reflexes a decrease of labelled iodine content in the thyroid was observed in most experiments. This indicated that cortical influences on the thyroid were realized under these conditions also.

In the light of our investigations it was imperative to consider the existing concepts of transmission of impulses from the CNS to the thyroid by direct neural pathways. If this were so, then neither removal of the hypophysis nor the subsequent removal of one adrenal and interruption of connection with the CNS of the other adrenal should impair the regulatory influence of the cerebral cortex on the thyroid. However, experiments showed that dogs deprived of the hypophysis and one adrenal and with denervation of the other adrenal showed complete disappearance of transmission of cortical influences to the thyroid. In these animals the thyroid reaction to unconditioned electrocutaneous stimulation also disappeared (Table 3).

TABLE 3

Effect of Electrodefensive Conditioned Reflexes on Radioactive Iodine Content of the Thyroid After Removal of the Hypophysis and the Right Adrenal and Transection of the Greater Splanchnic Nerve on the Left (Changes are Given as Percentage with Respect to the Initial Radioactivity)

Date of experiment	Change in radioactive iodine content of the thyroid						
	time (in minutes)						
	60	90	120	150	180	210	240
27/VI 1956	+4	+8	+12	—	+12	—	—
3/VII	+5	+5	—0	+5	+5	+10	+10
5/VII	0	—	—6	—	0	—	—
7/VII	+8	—	+8	—	+8	—	+8
9/VII	—4	—	0	0	0	—7	—
10/VII	0	—	+14	—	+7	—	+7
11/VII	+5	—	—5	—	+11	—	+11
13/VII	0	—	+4	—	+4	—	+6
14/VII	+5	—	+5	—	+2	—	0
10/VIII	—3	—	+6	—	—3	—	—
11/VIII	0	—	+9	—	+4	—	—
18/X	0	—	0	—	—3	—	—
20/X	—9	—	—3	—	—3	—	—
22/X	+4	—	+11	—	+11	—	—
After administration of thyrotropic hormone							
10/XII 1956	0	—	0	—	+8	—	—
11/XII	0	—	0	—	0	—	—
12/XII	0	—	0	—	0	—	—
15/XII	0	+11	—	—11	—	0	—
In experiments with electric reinforcement							
30/VI 1956	—11	0	0	0	—17	—11	—
25/X	—4	0	—8	—12	—8	—4	—
2/XI	0	—	+2	—	0	—	—
5/XI	—5	—9	—7	—3	—3	—	—
9/XI	0	11	—4	—	—	—	—
16/XI	+6	—	+6	—	0	—	+4

Note. Quadratic deviation of radioactivity value did not exceed 5%.

In addition, the experiments showed that in these animals there was depression of thyroid activity. It could be supposed that it was this depression which was the cause of the observed phenomena. To check this it was necessary to stimulate thyroid function in the operated animals and retest its reaction to conditioned reflexes against this background. The animals were given 4 ml thyrotropic hormone "Ambinon" (each ml containing 100 I.U.). Secretion of radioactive iodine by the thyroid increased after each administration of the hormone. Twice repeated administration of the hormone increased thyroid function almost threefold. Thus, before administration of the hormone the maximal absorption of radioactive iodine reached only 6% after 24 hours; after administration of the hormone maximal absorption of radioactive iodine was 17%. Experiments performed against the background of hormone-stimulated thyroid activity showed that fluctuations of iodine level in the thyroid under the influence of defensive conditioned reflexes in this case too were within physiologic limits; under these conditions the thyroid did not react to unconditioned electrocutaneous stimuli either (see Table 3).

These experiments give grounds for considering that following simultaneous severance of connection between the CNS and the hypophysis and adrenals cerebral cortical influences on the thyroid become impossible. The presence of either the hypophysis or the adrenals is consequently essential for mediation of cortical influences on the thyroid.

B. V. Aleshin and N. S. Demidenko [2] obtained definite reaction from the thyroid when they placed silver plates on the premotor area of the cerebral cortex in dogs both intact and deprived of the hypophysis. The authors regard changes in the thyroid in experiments with hypophysectomized animals as proof of direct influence on it of the nervous system. It is difficult to agree with these authors' conclusions since they do not take into account transmission of cortical influences by way of the adrenals. There is every reason to believe that transmission of cerebral cortical influences to the thyroid is effected by the adrenals in hypophysectomized animals and not by direct neural pathways as maintained by the authors cited above.

Our experiments thus demonstrate that the activity of the thyroid is dependent on regulatory influences of the cerebral cortex.

Cerebral cortical influences on the thyroid gland are mediated not only by the hypophysis but also by the adrenals.

SUMMARY

The effect of changes of the functional condition of the brain cortex on the changes of radioactive iodine content of the thyroid gland was studied. The functional condition of the cortex was changed by formation of the positive and inhibitory electrodefensive conditioned reflexes.

It was established that the influence of the central nervous system is transmitted to the thyroid gland by way of hypophysis or suprarenal glands. The regulating influence of the cerebral cortex does not reach the thyroid gland after removal of hypophysis and one of the suprarenal glands with complete interruption of connection between the central nervous system and the other suprarenal gland.

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PATHOLOGICAL PHYSIOLOGY AND GENERAL PATHOLOGY

REACTION OF THE CEREBRAL CORTEX AND OF THE HEART DURING OPERATIONS ON THE HYPOPHYSIS AND THE HYPOTHALAMIC AREA

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Physiologic investigations in the neurosurgical operating theater permit valuable studies of physiologic and pathologic features of the central nervous system on man, viz. functional connections between the cerebral cortex and subcortical formations, central regulation of the cardio-vascular system etc.

In order to study the functional cortical-subcortical connections electroencephalographic records were taken during operative removal of tumors (of the hypophysis, third ventricle and sella turcica) which is accompanied by stimulation of hypothalamic nuclei.

Some scattered references to studies on the influence of the hypothalamus on cortical potentials are available in the literature. Thus, stimulation of the hypothalamus "en masse" was noted to be associated with changes in cortical potentials in man [3].

In another communication [4] it was shown that during and after stimulation of the hypothalamus long-lasting changes in the spontaneous electroencephalogram could be observed; these could be interpreted as reflection of excitation since the EEG discharges on both hemispheres showed enhancement of both frequency and amplitude.

Lesions of the medulla do not alter substantially the electric activity of the cortex while lesions of the midbrain and diencephalon evoke gross bilateral changes in the EEG [2]. The author cited concludes from this that electrogenesis and, consequently, the functional state of the cortex depend both on the specific and non-specific projection systems. According to his data stimulation of the brain stem produces diffuse desynchronization of cortical potentials while destruction of the rostral parts of the brain stem leads to synchronization of slow waves and bursts of spindles.

The present investigation is concerned with the study of cortical reaction to hypothalamic stimulation in the course of surgical intervention for removal of tumors in this area.

EXPERIMENTAL METHODS

Those brain stem lesions were chosen for investigation in which the tumor focus requiring surgical intervention was found in the region of the sella, hypothalamus or optic chiasma. It is important that there is no primary involvement of the cortex in tumors of this localization. Since the very close connection between the hypothalamic area and cardio-vascular function is known from numerous experimental and clinical observations [1] simultaneous recording of the electrocardiogram was carried out together with the electroencephalogram. The material for the present work consisted of cases of pituitary adenoma (16 patients), third ventricle tumors (2 patients) and tumors in the sella turcica area (4 patients).

Reaction of the cortex at various stages of the operative procedure was studied by means of continuous recording of the bioelectric potentials from the posterior parietal and parietal areas of both hemispheres, i.e., cortical areas distant from the zone of neurosurgical intervention which used the frontal approach to the area of the sella and hypophysis with trephining in the frontal area and lifting of the base of the frontal lobe; scalp recordings were used.

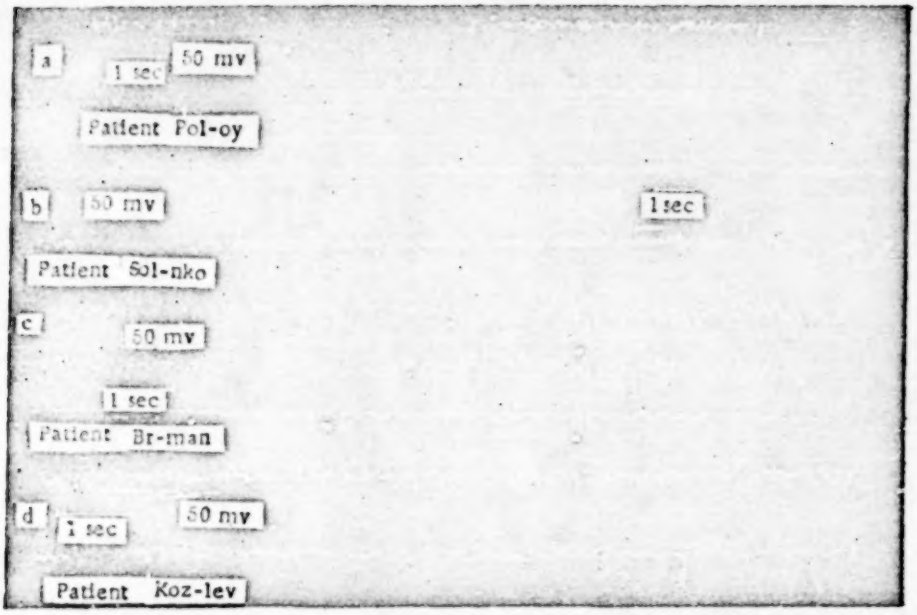

Fig. 1. Main types of electroencephalograms (a, b, c, d) recorded before operation in patients with pituitary tumors, at rest and with extraneous stimuli.
1) Right parieto-occipital area; 2) left parieto-occipital area.

The cortical potentials were led off by means of electrodes 8 mm in diameter and about 1 mm thick, made of pure tin. The electrodes were covered by a thin layer of a special paste ensuring minimal resistance between the electrode and the scalp. Bipolar leads proved to be the most reliable and convenient under operating theater conditions. The paste-covered electrodes were fastened to the scalp by strips of adhesive tape; they were placed over the occipital and parietal areas of both hemispheres.

In order to record the electroencephalogram and the electrocardiogram on the same strip a special combined electroencephalograph was used which was provided with automatic compensation of alternating current induction and required no screening of the theater. With the use of filters frequencies from 1 to 100 cycles per second could be recorded.

EXPERIMENTAL RESULTS

The main types of electroencephalograms observed prior to operation in patients with pituitary tumors both at rest and upon external stimulation are presented in Fig. 1. In 6 of 16 patients the original biopotential picture is dominated by a well formed, synchronous alpha rhythm of normal frequency and amplitude with marked reaction (diminution) to light stimuli (Fig. 1, a). In three patients the alpha rhythm was unstable with fairly frequent periods during which it was depressed. In these cases stimulation (such as prolonged clenching of fists) evokes, after a definite latent period, increased synchronization of the alpha rhythm, the latter disappearing upon cessation of stimulation (Fig. 1, b). In four patients fairly high voltage hypersynchronized

rhythm was seen over all areas of both hemispheres; this rhythm resembled the alpha rhythm in its appearance but differed from it in its properties since it was unchanged upon light and other, even rhythmic, stimulation, and did not usually exceed 7 to 8 cycles per second in frequency (Fig. 1, c).

This type of EEG is encountered more frequently upon extension of the tumor beyond the limits of the sella into supra- and parasellar areas. And, finally, when large pituitary tumors extending from the sella to the floor of the middle fossa or into the third ventricle are present considerable changes in the EEG consisting of complete or sustained absence of alpha rhythm and predominance of diffuse delta waves alternating, at times, with fast activity (Fig. 1, d) are seen.

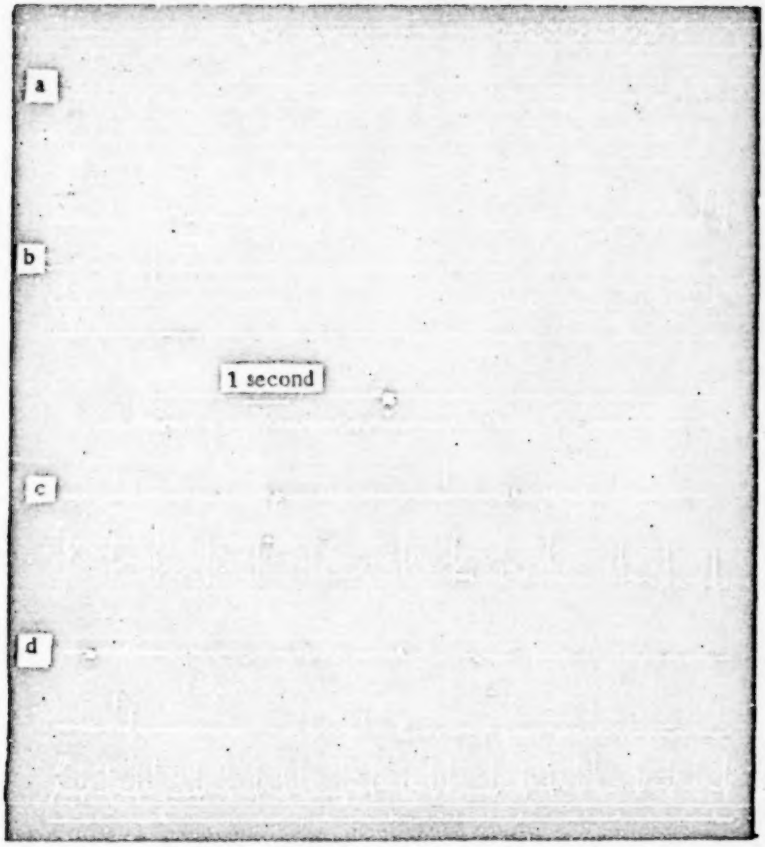

Fig. 2. Electroencephalogram changes in the posterior parietal areas and changes in the electrocardiogram upon stimulation of the hypothalamic region during removal of pituitary tumor.
a) Before trephining – normal depression of alpha rhythm on switching on a light; b) at the moment of stimulating the hypothalamic region with a tampon depression of alpha rhythm with subsequent slowing of the rhythm of cardiac contractions are noted; c) in another patient stimulation of the hypothalamic region is associated with bursts of fast activity on the contralateral hemisphere followed by definite slowing of cardiac contractions; d) the same in patient Sh-k suffering from encephalitis; upon the surgeon's approach to the basal ganglia the EEG shows bilateral bursts of fast activity with subsequent slowing of cardiac contractions.
Records from above down: electroencephalogram from the right occipito-parietal area, electroencephalogram from the left occipito-parietal area, lead I, time marker (1 second), also stimulus marker.

Results of simultaneous investigation of cortical and cardiac reactions during operative intervention in the hypothalamic region are presented in Figs. 2 and 3. In the case of patient N-v with a small pituitary adenoma a normal alpha rhythm, reactive to light, was recorded during the first stages of the surgical procedure. The electrocardiogram was also within normal limits (Fig. 2, a).

The next record (Fig. 2, b) shows a reaction consisting of alpha rhythm desynchronization and appearance of faster frequencies at the moment of stimulation of the hypothalamic region during removal of the pituitary tumor. Two seconds later a reaction is seen in the electrocardiogram when the rhythm of cardiac contractions is slowed appreciably (Fig. 2, b). The desynchronization reaction, i.e., cortical excitation, and slowing of cardiac contractions are in this particular case of short duration disappearing rapidly on cessation of hypothalamic stimulation.

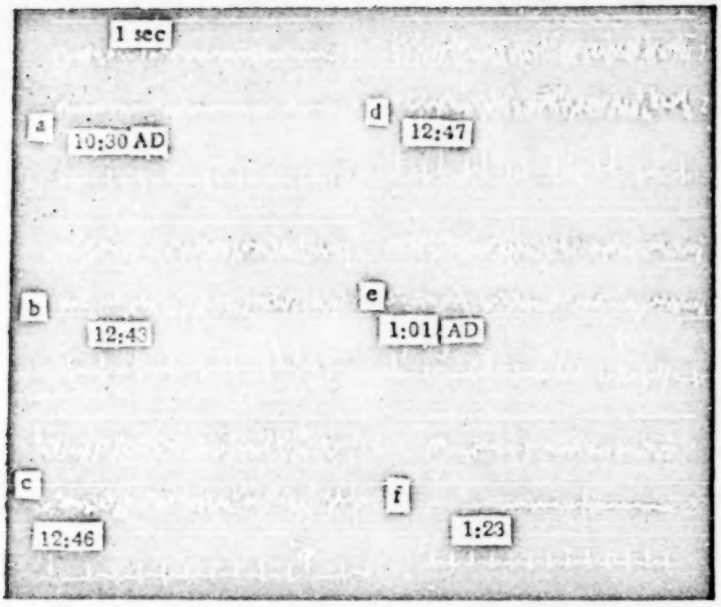

Fig. 3. Changes in the electroencephalogram and electrocardiogram at various stages of operative removal of pituitary adenoma under barbiturate anesthesia in patient M-na.
a) Before anesthesia; b) 4 minutes after administration of thiopental Na — increasing amplitude of fast activity; c) at the moment of removal of adenoma by suction, extrasystoles appearing after every 9 normal cardiac complexes; d) placing of tampon in the sellar region associated with sustained alternation of sinus and atrioventricular rhythms; e, f) gradual return to normal of the electrocardiogram toward the end of operation.
Records from above down: time marker (1 second); right occipito-parietal area; left occipito-parietal area, electrocardiogram, Lead I.

The next example illustrates even more clearly the appearance of cortical impulses and subsequent slowing of cardiac contractions at the moment of removal of the pituitary tumor (Fig. 2, c). In this case the corticopetal impulses evoke not desynchronization of the alpha rhythm as was the case in the preceding patient, but the appearance of bursts of higher voltage impulses on the contralateral hemisphere following which slowing of cardiac contractions becomes clearly visible in the electrocardiogram.

When more pronounced changes in the original electroencephalogram are present in the case of large tumors extending into the middle fossa stimulation of the hypothalamic region during removal of the tumor is seen to be accompanied by a more prolonged reaction of cortical excitation in the form of frequent sharp waves. This reaction was coincident with a change from normal frequency of cardiac contractions (80 per minute) to prolonged slowing – down to 48 per minute. In these cases, in addition to slowing of cardiac contractions, it was noted that extra auricular contractions were occurring. Such cortical reactions followed by slowing of cardiac activity were observed not only during intervention in the sellar area but also upon temporal lobe approach to the basal ganglia (Fig. 2, e).

In the example cited, stimulation of the basal ganglia was carried out against the background of ether anesthesia (to prevent hyperkinesis). Bursts of cortical discharges appear in response to stimulation of the hypothalamic region against the background of lowered amplitude of spontaneous cortical activity (as the result of ether anesthesia). Attention is drawn to the fact that the cortical reaction occurs prior to sinus bradycardia; this may indicate transmission of excitation from the cortex to the vagal center.

The neurosurgeons removed some pituitary tumors under barbiturate anesthesia which is usually used after examination of the optic chiasma and the sella (Fig. 3). Stimulation of the hypothalamic region under barbiturate anesthesia is not clearly reflected, electrographically, in the cortex since during the first stage of anesthesia, particularly after administration of thiopental sodium, sustained stimulation is observed; this is expressed electroencephalographically by high voltage fast activity (16-22 cycles per second) recorded continuously over a period of twenty or more minutes. Similar EEG pictures were seen under the influence of barbiturate in patients in whom no pathologic focus was discovered.

Considerable changes in cardiac reactions are seen against the background of general anesthesia. Thus, upon introduction of a tampon into the sellar region after removal of the tumor sustained heterotopy was observed. Analysis of the dynamics of the electrocardiogram (Fig. 3) revealed that foci of excitation arise at the same time in the atrioventricular node and the bundle of His together with excitation emanating from the sinus node.

It may be supposed that these manifestations of heterotopy under conditions of general anesthesia are caused by direct influence of subcortical impulses on the functional properties of nervous apparatus and conducting system of the heart. The rapid reversal of these changes in cardiac activity (Fig. 3, e, f) indicates, from our point of view, adequate functioning of the cardiac muscle itself and of coronary circulation.

The comparison of electroencephalographic and electrocardiographic data obtained simultaneously during operative removal of pituitary tumors thus permits the study of cortical and cardiac reactions to hypothalamic stimulation in man. Cortical reactions to stimulation of the hypothalamic zone are expressed electrographically by desynchronization of the rhythmic potentials lasting for 3-5 seconds and longer. During lowering of alpha rhythm amplitude upon hypothalamic stimulation bursts of sharply outlined waves are observed. One to two seconds after the reaction of cortical excitation changes in the direction of sinus bradycardia appear in the electrocardiogram lasting from two to three seconds to several minutes.

Such cortical reactions associated with subsequent slowing of cardiac activity were also observed during manipulation in the region of the basal ganglia. When the hypothalamic region is stimulated against the background of barbiturate anesthesia the cortical reaction is often not reflected clearly in the electroencephalogram owing to predominance of sustained stimulation in the cortex under the influence of the first stage of barbiturate anesthesia. At the same time stimulation of the hypothalamus under these conditions is reflected in the electrocardiogram by a picture of heterotopy; in addition to excitation proceeding from the sinus node, foci of excitation arise in the atrioventricular node and the bundle of His.

SUMMARY

Data concerning the study of electroencephalograms (EEG) and electrocardiograms (ECG) are presented in this paper. These EEG and ECG were taken simultaneously during surgical removal of the tumors of hypophysis.

Investigations which were carried out on 22 patients show that reactions of the cortex to stimulation of the hypothalamic area may be demonstrated electrocardiographically by desynchronization of the rhythm of potentials or in appearance of "acute" potentials. These changes of ECG are especially pronounced if there is a decreased amplitude of the alpha rhythm in the initial EEG.

Similar manifestations of the cortical reactions, associated with the following sinus bradycardia, were revealed in manipulations in the area of the basal ganglia.

Transitional heterotopia was observed in the ECG of 5 patients in stimulation of the hypothalamus on the background of barbiturate narcosis.

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RESPONSE OF ADRENAL CORTEX OF NEW-BORN RATS TO STRESS STIMULI

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In the literature are indications that a stress response can be observed in rats by about the 13th to 14th post-natal day [1, 2, 3, 5, 9]. The question as to the time when the adrenal cortex in the new-born first begins to participate in the accommodating reactions of the organism has a considerable interest when discussing the physiologic characteristics of the early post-natal period.

The present investigation had for its purpose a more precise answer to the problem just presented. As a criterion of adrenal cortical response we employed the well-known eosinopenic phenomenon which follows secretion of cortical hormones.

EXPERIMENTAL METHODS

To stimulate secretion of cortico-steroids, we employed painful electrical current skin shocks and also intraabdominal injections of adrenalin. Electric shock was produced in a cage having a floor made of metallic rod electrodes so that any position of the animal would close the circuit. Alternating current at 30-40 volts for three minutes was utilized. The adrenalin was used in the proportion of 0.05 mg per 100 g weight of animal. Studies were made from blood drawn from the central vein of the tail and from the heart. Eosinophile counts were made by the direct method [4, 6, 7, 8]. This consists of taking up the blood flowing from the wound directly into the mixing pipette and quickly diluting it 20-fold with a water solution of acetone with eosin. Altering slightly the usually recommended concentrations, we used for the blood of adult rats a 20-30%, while for the blood of the new-born a 30-50% solution of acetone. The eosin content in both instances was 2%.

With the stated acetone concentrations, all the formed elements with the exception of the eosinophiles are destroyed. Under such conditions, even a slight increase in the number of eosinophiles is detected readily by the red granulation and they can be counted by the usual method in the Goryaev chamber as they float among a few other surviving cell elements.

The adult animals were tested routinely as follows: the normal eosinophile count was determined, this being followed by the action stimulating the cortex and then, four hours later, the eosinophile count was repeated. In new-born rats, blood could be obtained only from the heart, this necessitating the sacrifice of the animal. For this reason, the stress effect was determined in a different manner. The animals of each litter were divided into two groups. The members of one group were not subjected to any stress and their eosinophile counts were taken as "base" level. The second group had a stress stimulus following which, four hours later, their eosinophile counts were taken and then the two groups were compared. The adrenal response to stress was measured in terms of the diminished eosinophile count in the second group as compared with the first. Beginning with the sixth day of life, blood was obtainable twice and so the usual method of testing was possible.

EXPERIMENTAL RESULTS

The number of eosinophiles present in the early post-natal period is much less than in adult animals (see graph). In rats within the first 5 days of life the values vary from 30 to 130 cells per cc of blood while in the adult animals the control figures vary from 150 to 700 eosinophiles per 1 cc of blood.

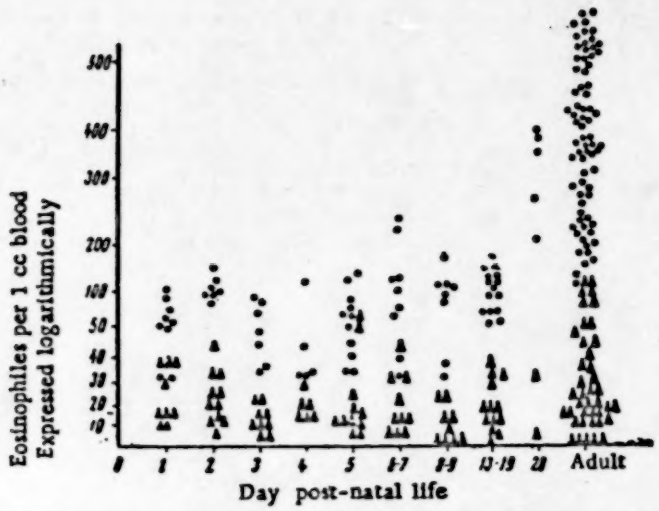

Figure showing graph of eosinophiles present normally and after "stress".
Dots (...) normal number eosinophiles; triangles (▲▲▲) number eosinophiles 4 hours after the stress (stimulus, adrenalin injection).

The Eosinopenic Reaction in Rats of Varying Ages

Average size in terms of percentage of the decrease in the number of eosinophiles in animals subjected to "stress"				
age in days	stimulation with electric current		adrenalin injection	
	No. exp.	% change	No. exp.	% change
1	6	−50	3	−76
2	6	−73	5	−76
3	6	−73	3	−80
4	4	−58	1	−65
5	7	−63	4	−74
6-7	4	−84	3	−87
8-9	7	−92	2	−90
13-19	8	−78	4	−93
28	1	−98	1	−91
Adults	22	−90	18	−89

The graph makes it evident that a response to "stress" is present from the very first day of life. In the experimental group of rats within the first five post-natal days, the eosinophile count falls as a response to stress an average of 10-40 cells per 1 cc or, in other words, in 50 tests a drop of 58% from base level. This is somewhat lesser fall than in adult animals in whom, in 40 tests, there was a decrease of 90%. In older rat young,

the reaction becomes more pronounced reaching the level seen in adults in the second 10 days of post-natal life. The corresponding figures are shown in the table; from it can be seen that the eosinopenia following adrenalin given to both adult and new-born animals is almost identical with the response to the stress of an electric shock.

These experiments demonstrate that the adrenal cortex is active from the moment of birth and participates immediately in the responses of the organism to "stress". This means that this mechanism, so important for adaptation to external influences is functionally capable, relatively speaking, of performing its functions from the first day of the animal's existence.

Our disagreement with the results obtained by other authors, who failed to record adrenal activity until the 14th day of life, is apparently due to differences in registering methods. Our procedure, undoubtedly, is a more sensitive recorder of the eosinopenic response to corticosteroids than the morphologic indicators employed by other authors [1, 2, 5].

SUMMARY

The reaction to stress was studied in rats of various ages. It was demonstrated that from the very first day of post-natal life there is an eosinopenic reaction following stimulation by electric current and intra-abdominal introduction of adrenalin.

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CERTAIN REFLEXES OF THE REINNERVATED TRANSPLANTED KIDNEY

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(Presented by Active Member of the Acad. Med. Sci. USSR V. N. Chernigovsky. Received July 26, 1957)

The reflex influences of the kidney, pelvis and ureter upon the functions of various organs and systems have been investigated by many authors.

N. P. Simanovsky [5], observed reflex alterations in blood pressure and cardiac rhythm when the pelvis and ureter were stimulated. V. V. Zakusov [2] studied reflex influences of the kidney vessel interoceptors using the perfusion method and came to the conclusion that only high concentrations of nicotine and aconite could alter reflexly the blood pressure and respiration. V. N. Chernigovsky [7] gave decisive proof in favor of the existence of kidney interoceptors when he showed that such substances as acetylcholine, carbonic acid and similar substances produced reflex alterations in blood pressure and respiration when they were present in concentrations approaching their usual physiological levels.

V. N. Chernigovsky, as well as O. S. Merkulova [3] and I. P. Nikitina [4], performed acute studies only. Relatively little has been done concerning the role of interoceptors of the kidney in the intact organism.

G. M. Shpuga [8] proposed a method for restoring the innervation of the intact kidney after it had been transplanted, the procedure being to anastomose the central end of the vagus-sympathetic trunk in the neck to the peripheral ends of the kidney nerves. This method has been used to study the role of efferent nerves in urinary formation [9] and also permits an approach to an investigation of reflexes arising from the kidney interoceptors under the conditions of a chronic experiment.

EXPERIMENTAL METHODS

In five dogs an autotransplantation of a kidney was performed, it being moved to the neck region. The right kidney was transplanted to the cervical region, the right carotid artery being anastomosed to the renal artery and the right jugular vein being joined to the renal vein. After the circulation in the transplanted kidney had been restored, the central end of the right vagal sympathetic trunk was joined to the peripheral end of the kidney nerves. The nerves were sewn end to end, a fine caproned thread being used for the one or two ties. In this manner, conditions were set up permitting the vagus to grow into the transplanted kidney parenchyma. The kidney thus became reinnervated, the nerves lost during the transplantation becoming replaced. It should be observed that the new innervation is materially different from that possessed by the intact kidney.

EXPERIMENTAL RESULTS

Following one or two months after the transplantation, in the experimental dogs the following phenomenon developed: light massage of the transplanted kidney evoked coughing; a similar effect was produced when the transplanted kidney was tapped lightly with the handle of a scalpel.

In the first experimental series we studied respiratory changes produced by mechanical irritation of the transplanted, reinnervated kidney as well as the changes produced by stimulating the kidney parenchyma by means of an induction current. In the first 2-3 months after the transplantation, mechanical irritation of the kidney produced the following stages of respiratory alterations. First, mechanical stimulation of the kidney increased the depth of respiration, this being followed by individual cough impulses and, finally, there would be a fit of coughing. After 3-4 months following the operation the coughing attack would be succeeded by a retching movement which would not, however, lead to any production of vomitus.

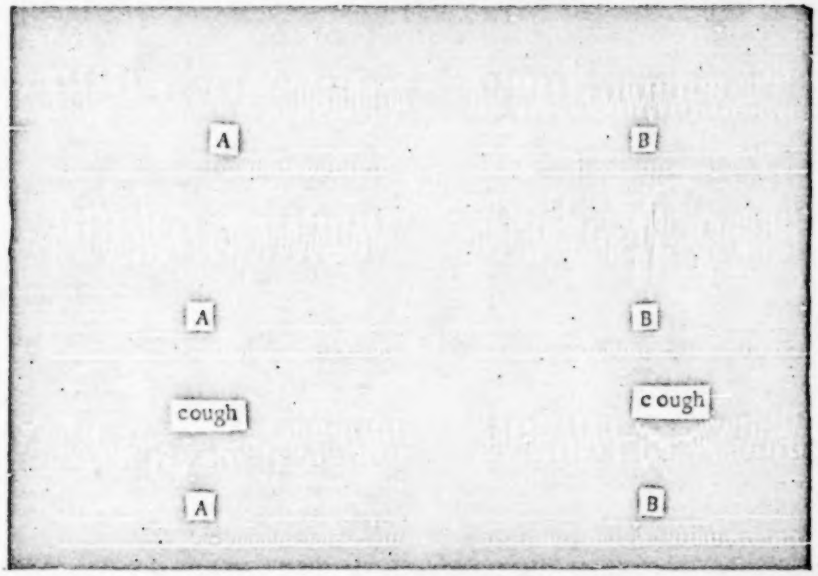

Fig. 1. Respiratory changes induced by kneading (A) and tapping (B) the reinnervated kidney of the dog Bulb. I) After 39 days; II) after 48 days; III) 53 days after transplantation of the kidney and restoration of the innervation.
Significance of tracings (from above): respiration, stimulation, time record (2 seconds).

The cough, arising from tapping the kidney, appeared sooner after the operation of transplantation than did the cough due to massage (Fig. 1).

Control experiments in which the skin and muscles around the kidney were stimulated proved that there would be coughing only when the kidney itself and not the surrounding tissues were stimulated. These experiments indicate that it was the vagus that reinnervated the kidney.

The cough reflex which we observed when we stimulated the transplanted kidney which had become reinnervated by the vagus nerve is readily understood when the experiments of P. K. Anokhin and A. Ivanova [1] are studied.

The parenchyma of the reinnervated transplanted kidney was stimulated by means of an induction current. The parenchyma of the transplanted reinnervated kidney was pierced by needle electrodes introduced through the overlying skin and connected to the secondary coil of the induction apparatus. When the intensity of the stimulus was increased, the following events were observed. Cough appeared first, this being followed with increasing current strengths by typical retching and raising of vomitus (Fig. 2).

Comparing the results of this experiment with the data of other workers [2], we can affirm that induction current stimulation of the parenchyma of the transplanted, reinnervated kidney produces more clearly evident reflex response than does stimulation of the intact kidney.

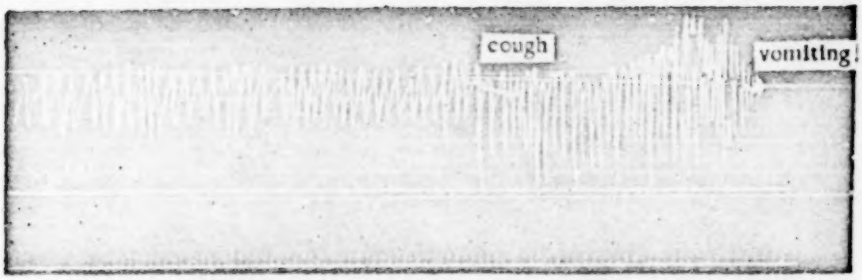

Fig. 2. Respiratory changes induced by stimulating with induction current (obtained from transformer apparatus, voltage in first coil being 4 V with a frequency of 60 stimuli per minute) the parenchyma of the transplanted reinnervated kidney. Significance of tracings as in Fig. 1. The figures in centimeters the distance of the coils in the sled apparatus.

Thus, the first series of experiments indicates that mechanical and electrical stimulation of the parenchyma of the transplanted, reinnervated kidney causes irritation of its interoceptors which manifests itself by corresponding reflex reactions.

In the second series of experiments we explored the reflexes in the transplanted, reinnervated kidney when its pelvis and ureter were stimulated mechanically and chemically.

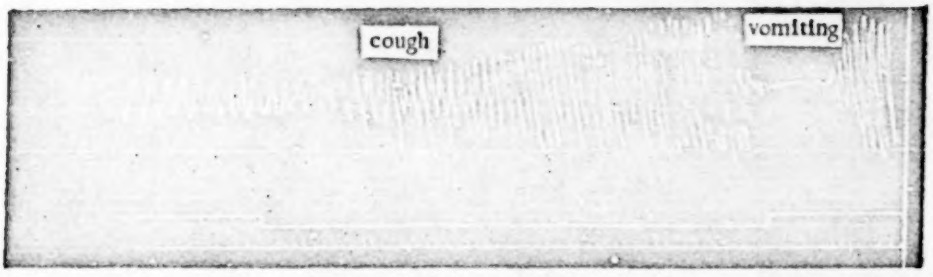

Fig. 3. Respiratory changes when the pelvis of the transplanted, reinnervated kidney was distended by the pressure of the contained fluid.
Significance of tracings the same as in Fig. 1.

To stimulate mechanically the pelvis and ureter we employed the following method. With the aid of a fine ureteral catheter we introduced into the pelvis a solution of physiological saline. A soft intestinal clamp was placed at the ureteral orifice stopping outflow of fluid from the pelvis and ureter.

Distension of the pelvis and ureter under a pressure of 80-100 mm mercury produced in the first experiment coughing and vomiting (Fig. 3) but, when the pelvis was distended the second, third, and fourth times, cough also was produced but vomiting could be induced only with considerably higher, more prolonged

periods of distension and, in further experiments, could not be induced at all. Still later, succeeding experiments could not evoke coughing at all, the distension of the pelvis and ureter causing typical movement reactions on the part of the animal accompanied by increase in amplitude and rate of the respiratory movements, i.e., an illness type of reaction.

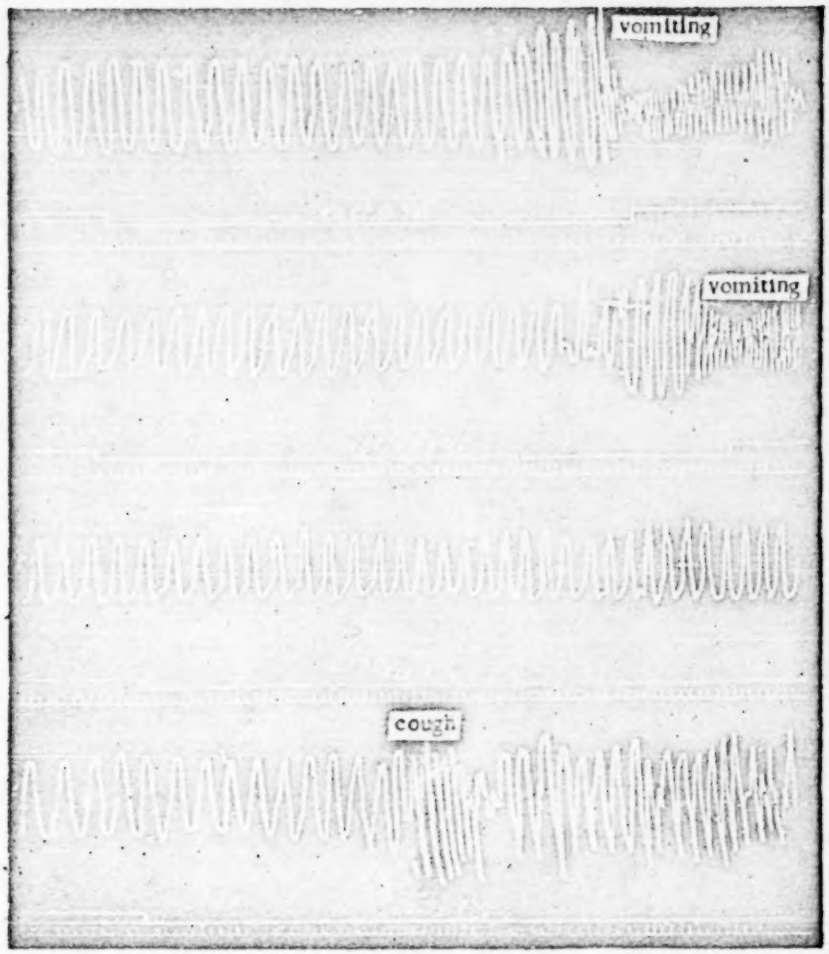

Fig. 4. Changes in respiration induced by irrigation of the pelvis of a transplanted, reinnervated kidney with chlorides of sodium, magnesium, potassium and calcium. Significance of tracings the same as in Fig. 1.

These experiments seem to indicate that continued pelvic irritation in the transplanted, reinnervated kidney changes the vagal responses – cough and vomiting – by a reaction of illness. The disappearance of this "vagal" response to systematic stimulation of the pelvis and ureter may be due to a "transformation" [1] of the center or, possibly, an adaptation of the pelvic interoceptors to continuing irritation.

In further experiments we decided to observe whether there would be a response on the part of the pelvic interoceptors of the transplanted reinnervated kidney to chemical stimuli.

The pelvis of the transplanted, reinnervated kidney was irrigated through the ureteral catheter with various fluids which were permitted to flow out freely. Control experiments on the transplanted, reinnervated kidney using normal saline showed that respiration was unaffected.

The greatest respiratory alterations were observed when the pelvis was irrigated with various salt solutions. Sodium chloride in 5-10% solutions induced vomiting; smaller concentrations did not materially affect respiration. Vomiting was also induced by a 2.5% solution of magnesium chloride. When the pelvis was irrigated by potassium chloride solutions as weak as 1%, the amplitude and frequency of the respirations was increased. It is interesting that potassium chloride in concentrations as high as 5-10% produced neither cough nor vomiting. Beginning with 1% concentrations, irrigation of the pelvis with calcium chloride produced definite increase in the amplitude and frequency of the respiratory movements. In Fig. 4 is seen the effect of a 1% solution.

When the pelvis was irrigated with solutions of ammonia and glucose, even in concentrations as strong as 10%, respirations were unaffected, the same being true of solutions of acetylcholine and adrenalin (1: 10,000).

It is evident from these experiments that the interoceptors of the pelvis are most sensitive to saline solutions, as some of these evoke effects in concentrations as weak as 1%. However, these results do not mean that the pelvic interoceptors play an important role in normal physiological surroundings as relatively high concentrations of the salts are required to stimulate them.

Comparison of experiments performed in irrigating the pelvis of the transplanted, reinnervated kidney with those made on the intact kidney leads to the conclusion that this is a valid method for examining those interoceptor reflexes which cannot be studied under normal conditions.

This method of reinnervating the transplanted kidney permits a study of interoceptor influences existing in the vessel stream under chronic experimental conditions. Our experiments are only preliminary. Into the artery going to the reinnervated transplanted kidney a 2% solution of sodium chloride injection would induce coughing, while a 0.5% solution of potassium chloride would result in increased frequency of respirations. Control experiments injecting similar solutions into the jugular vein did not alter respirations. This must mean that the injection into the artery leading to the transplanted, reinnervated kidney stimulated the interoceptors of the vessel bed in the kidney. It is probable that this reinnervation will permit kidney perfusions in chronic experiments.

DISCUSSION OF RESULTS

The experiments described above indicate the presence of reflex mechanisms when the parenchyma of the transplanted, reinnervated kidney is stimulated mechanically or electrically, and when the pelvis is stimulated mechanically or chemically. These effects are more evident than when the intact kidney is stimulated in a parallel manner.

This lesser reflex response on the part of the intact kidney may be due to the fact that the stimulus spreads diffusely through the entire nervous system [7, 3, 4, 6]. As these authors have shown, under specific conditions reflexes from kidney interoceptors affect blood pressure, respiration, the skeletal musculature and lymphatic circulation.

The impulses arising during stimulation of the transplanted, reinnervated kidney interoceptors enter the vagus nerve by way of its afferent pathways and flow to the respiratory, coughing and vomiting centers. In the experimental animals we observed a very sensitive response of the respiratory center to the most varied stimuli to the interoceptors of the transplanted, reinnervated kidney. It may well be that other systems are also affected. It is only the simplicity and proven sensitivity of the respiratory system that made us confine our studies to just this indicator.

It must be true that the respiratory, cough and vomiting centers respond in their specific manner when the interoceptors of the transplanted, reinnervated kidney are stimulated; while this limits somewhat the investigative procedures, it does permit a clarification as to just which stimuli and to what degree will affect the interoceptors of the transplanted, reinnervated kidney.

SUMMARY

The right kidney was transplanted in 5 dogs, being moved to the cervical region. The vagus was anastomosed to the nerve trunk leading to this kidney. The afferents grew in from the kidney so that mechanical and chemical, as well as electrical stimulation of the kidney parenchyma or the kidney pelvis would lead to respiratory alterations or affect the vomiting and cough centers.

Results, so far, are preliminary in nature and further studies will be undertaken.

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BIOCHEMISTRY AND BIOPHYSICS

S^{35}-METHIONINE UPTAKE BY PROTEINS OF THE STRUCTURAL ELEMENTS OF TISSUE CELLS IN IRRADIATED RATS

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(Received May 24, 1957. Submitted by Active Member of AMN SSSR V. N. Orekhovich)

Disturbances of protein metabolism in the structural elements of tissue cells are known to occur when the animal organism is exposed to ionizing radiation in doses sufficient to produce acute radiation sickness. These disturbances are characterized by quantitative changes in the protein content of the nucleus and cytoplasm, changes in the properties of desoxyribonucleoprotein and desoxyribonucleic acid [2, 3, 4] and changes in antigenic properties of the tissues [5, 6].

In connection with this it appeared important to study the level of S^{35}-methionine uptake by cell proteins of irradiated animals since such uptake reflects to a certain degree the state of synthetic processes taking place. We were interested not in tissue proteins en masse but in individual proteins of isolated cellular microstructures.

The problem with which the present communication is concerned is the study of radioactive methionine uptake by proteins of cell nuclei (desoxyribonucleoprotein, acid and "residual proteins"), mitochondria and microsomes (water-soluble ribonucleoproteins, lipoproteins) and hyaloplasma in the liver and small intestine mucosa of rats with acute radiation sickness.

EXPERIMENTAL METHODS

Examination of the experimental group of rats for radioactive proteins was carried out three days after irradiation of the animals with roentgen rays and three hours 30 minutes after administration of labelled methionine. The control group of animals was also examined for radioactive proteins 3 hours 30 minutes after administration of S^{35}-methionine. The latter was always given subcutaneously in doses of 0.5 ml calculated on 3 μC per 1 kg body weight.

Experiments were performed on white male rats (total of 150 animals) weighing 190-210 g which were kept fasting for 12 hours preceding the experiment and were only allowed water. Determinations were carried out on homologous tissues from 10-20 rats.

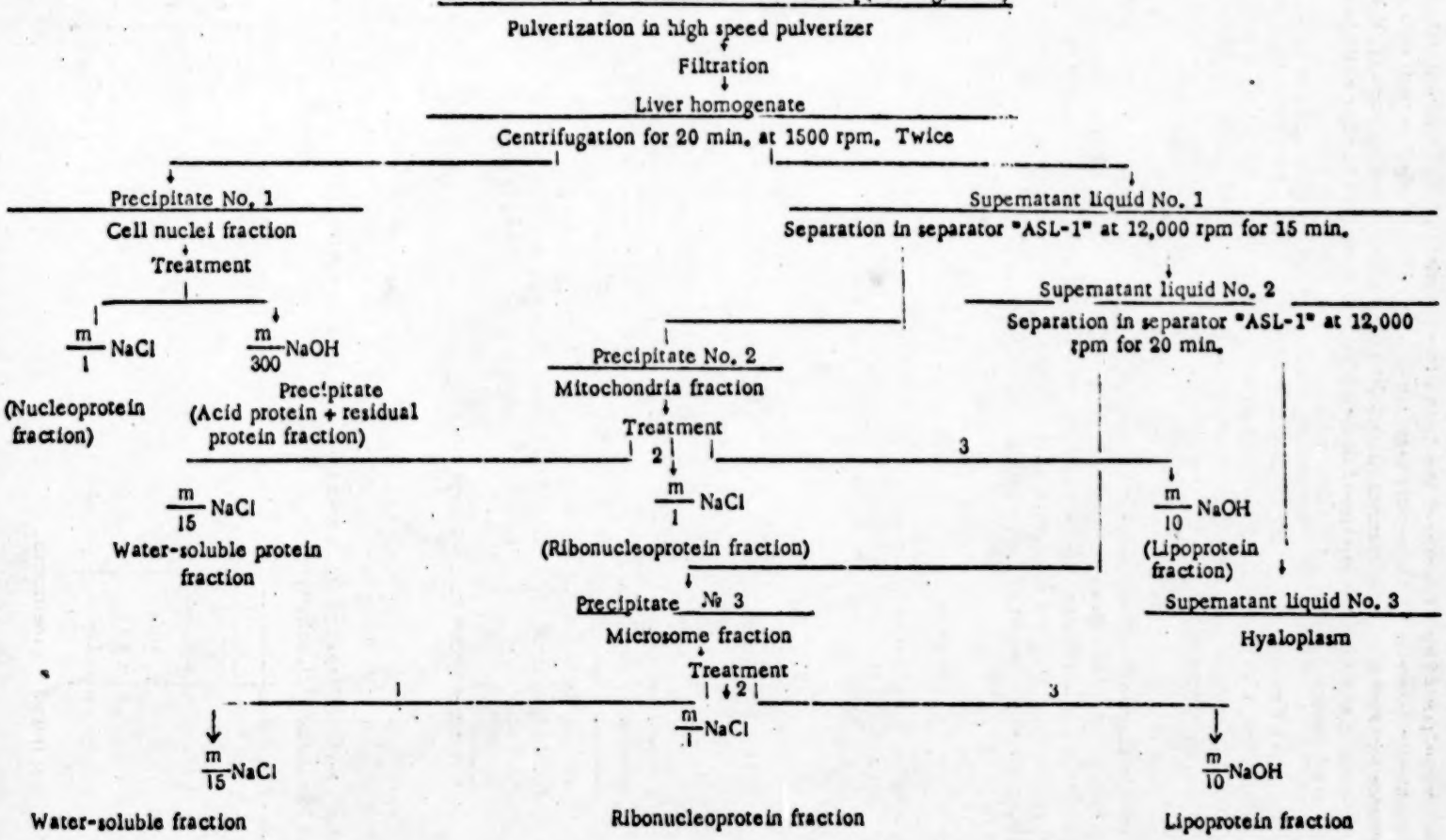
The animals were subjected to a single total irradiation with a voltage of 180 kV, current of 15 ma, focal distance of 30 cm, using copper (0.5 mm) and aluminum (1 mm) filters. The summated dose per rat was equal to 800 r. Under the given conditions of irradiation all the animals died within 7 days. The highest incidence of death was observed on the 5th day (80%).

A Dounce's [7] method was used for isolation of cell nuclei. Fractionation of nuclear proteins was carried out by the method of I. B. Zbarsky and S. S. Debov [1].

The cytoplasmic structures – mitochondria, microsomes and hyaloplasm – were isolated according to the scheme presented below. This diagram also shows the scheme for fractionation of the microstructures into separate proteins.

SCHEME

for isolation of structural elements of the cell and fractionation of proteins from these microstructures



Isolation of solid protein preparations and determination of their radioactivity were performed as follows. The proteins were precipitated from corresponding solutions (see scheme) with 20% trichloracetic acid, washed three times with 5% trichloracetic acid to remove radioactive methionine adsorbed on their surface and rendered lipid-free by thrice repeated successive treatment with 96° alcohol, alcohol-ether mixture (1:3) and ether. The protein preparations so obtained were exposed to the air until no more odor of ether could be detected and were then shifted through a sieve.

Radioactivity was determined on 10 mg dry protein placed on a stainless steel disc with a surface measuring 2 cm^2. A fine, maximally uniform layer of protein was obtained as follows. The weighed protein sample was carefully distributed over the whole surface of the disc by means of a glass rod. Then 5-6 drops of ether was added carefully to the protein from a pipet from the edge of the disc and the whole stirred by rotary movement.

Slow, uniform evaporation of ether took place under these conditions and the protein particles suspended in it were precipitated over the whole surface of the disc in a smooth, fine layer. The advantage of this method of preparation of "targets" as compared to the use of aqueous alcohol lies in the rapidity of the procedure (2-3 minutes) and the formation of a uniform layer of protein. Radioactivity counts were carried out with the use of installation B with a thin-walled counter.

EXPERIMENTAL RESULTS

Data illustrating the effect of roentgen rays on the uptake of radioactive methionine sulfur by the proteins of cell nuclei and cytoplasm are provided by results obtained for normal and irradiated rats and are presented in Tables 1-3.

TABLE 1

S^{35}-Methionine Uptake by Proteins of Cell Nuclei in Liver and Mucosa of Small Intestine in Normal and Irradiated (800 r) Rats (Expressed as Percentage of Introduced Radioactivity) *

Nuclear proteins	Liver		Small intestine mucosa	
	normal	experimental	normal	experimental
Nucleoprotein	2.03	3.22	5.20	5.54
Acid	3.31	4.83	6.93	9.76
"Residual"	1.23	2.45	2.01	1.67

* Average figures from 4-5 experiments.

TABLE 2

S^{35}-Methionine Uptake by Hepatic Cytoplasmic Proteins of Normal and Irradiated (800 r) Rats (Expressed as Percentage of Introduced Radioactivity) *

Cytoplasmic proteins	Mitochondria		Microsomes		Hyaloplasm	
	normal	experimental	normal	experimental	normal	experimental
Total					0.98	0.95
Water-soluble	0.86	0.90	0.80	0.85	–	–
Ribonucleoproteins	1.58	0.95	1.55	0.34	–	–
Lipoproteins	1.48	1.05	1.43	1.15	–	–

* Average figures from 3-5 experiments.

Table 1 shows that radioactivity of nuclear proteins in the liver of irradiated rats is higher than in normal rats. As regards nuclear proteins in cells of the small intestine mucosa, definite increase of labelled methionine uptake is observed only in the acid protein. Radioactivity of nucleoprotein and "residual" protein remains within normal limits in this case.

Data presented in Table 2 indicate that irradiation is associated with substantially decreased S^{35}-methionine uptake by the ribonucleoprotein and lipoprotein fractions of hepatic cytoplasmic proteins; the greatest depression of uptake is seen in ribonucleoproteins of the microsomes (down to 22% of normal). The level of S^{35}-methionine uptake by water-soluble mitochondria and microsome proteins and hyaloplasm proteins remains within normal limits.

Unlike the liver, the cytoplasmic microstructures in the small intestine mucosa of irradiated rats show slower S^{35}-methionine uptake by all the proteins studied, including water-soluble ones. This is most pronounced in the ribonucleoprotein fraction from mitochondria and microsomes (Table 3).

TABLE 3

S^{35}-Methionine Uptake by Cytoplasmic Proteins in Small Intestine Mucosa of Normal and Irradiated (800 r) Rats (Expressed as Percentage of Introduced Radioactivity) *

Cytoplasmic proteins	Mitochondria		Microsomes		Hyaloplasm	
	normal	experimental	normal	experimental	normal	experimental
Total	–	–	–	–	1.80	1.40
Water-soluble	2.55	1.45	2.80	1.30	–	–
Ribonucleoproteins	2.30	0.90	2.00	0.60	–	–
Lipoproteins	2.43	1.35	2.10	1.00	–	–

* Average figures from 3-5 experiments.

Roentgen irradiation thus leads to increased uptake of radioactive methionine sulfur by nuclear proteins of hepatic cells and by acid protein of intestinal mucosa. S^{35}-methionine uptake by desoxyribonucleoprotein and "residual" protein of the nuclei in intestinal mucosa is unchanged as compared to the normal. The uptake of radioactive methionine sulfur by the cytoplasmic proteins of the liver and intestinal mucosa is slower in irradiated rats than in normal ones.

SUMMARY

The rapidity of inclusion of S^{35}-methionine into the cells of various tissues was studied in rats in acute radiation sickness. Inclusion of S^{35}-methionine was studied in the proteins of cellular nuclei (desoxynucleoprotein, acid and "residual"), mitochondria and microsomes (water-soluble ribonucleoproteins, lipoproteins) and hyaloplasm of the liver and of the mucous membrane of the small intestine.

It was established that x-ray irradiation causes increased inclusion of radioactive sulfur methionine into all proteins of the cellular nuclei of the liver, as well as in the acid protein of the mucous membrane of the small intestine. Inclusion of S^{35}-methionine into the desoxyribonucleoprotein and "residual" protein of the nuclei in the mucous membrane of the small intestine does not change in comparison with the same in normal conditions.

Decreased inclusion of radioactive sulfur methionine is noted in cytoplasmic proteins of the liver and in the mucosa of the small intestine. This decrease is especially pronounced in ribonucleoproteins of mitochondria and microsomes.

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THE INFLUENCE OF VITAMIN A ON NUCLEIC ACID CONTENT AND PROTEIN SYNTHESIS IN THE ORGANISM

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Lesions characteristic of A-avitaminosis are keratinization of cellular elements, degeneration of lachrymal glands and mucous membranes, epithelial cornification in the lungs, intestine, renal pelvis, urinary bladder, uterus and ovaries. In the absence of vitamin A the process of transformation of columnar epithelium into squamous is impaired. This indicates that vitamin A is essential, above all, for the cells of epithelial tissue where processes of regeneration play a particularly important part [1].

V. N. Bukin and L. Ya. Areshkina [2] used fractional separation of hydrolysate from fish livers to show that the bulk of vitamin A contained in the liver was bound with proteins. This combination with proteins is in the nature of adsorption or association rather than chemical linkage and is fairly easily disrupted.

Our work has shown that deposition of vitamin A in the liver of white rats depends on the amount of protein in the diet: increasing the amount of protein leads to increased deposition of vitamin A in the liver; decreasing the amount of dietary protein (below normal) is accompanied by depletion of vitamin A stores [3]. Moreover, when the diet is deficient in protein the transformation of carotene into vitamin A in the organism is impaired.

These data, as well as the effect of vitamin A on growth and on tissue regeneration suggested the question concerning the influence of vitamin A on the synthesis of protein in the organism, particularly in the liver. The connection observed between protein synthesis and nucleic acids suggested the need to determine the content of the latter in liver tissue under conditions of vitamin A deficiency.

EXPERIMENTAL METHODS

The content of ribonucleic acid and desoxyribonucleic acid in rat liver was determined by the Thannhauser and Schmidt method modified by O. P. Chepinoga et al. (based on the amount of ribonucleic acid and desoxyribonucleic acid phosphorus) [4]. The experiments were performed on white male rats. The initial weight of animals was 50 g. The experimental animals were given the usual synthetic diet, free from vitamin A, while the control animals received the same diet but with addition of vitamin A.

EXPERIMENTAL RESULTS

Table 1 shows the content of ribonucleic acid and desoxyribonucleic acid in the liver of animals with A-avitaminosis and of control animals. The former show increased content of both acids. Parallel with this,

the content of total phosphorus in the fraction containing ribonucleic acid, desoxyribonucleic acid and nucleoproteins was determined in the liver of animals with A-avitaminosis. These data are presented in Table 2.

TABLE 1

Ribonucleic Acid and Desoxyribonucleic Acid Content of Liver in Animals With A-Avitaminosis and in Normal Animals (in mg %)

No.	Experiment		Control	
	DNA	RNA	DNA	RNA
1	51.2	142.0	24.8	149
2	51.0	136.0	20.8	147.8
3	42.0	174.0	18.2	144.0
4	31.0	166.0	26.9	178.0
5	31.0	185.0	20.9	148.0
6	34.0	181.0	21 8	143.3
7	43.3	169.0	25.6	106.0
8	46.2	200.9	23.5	141.0
9	50.0	161.9	24.6	144.0
10	42.0	166.2	26.9	150.9
11	39.6	—	—	167.4
12	37.3	—	—	160
13	41.0	—	—	—
Average	41.5	168.2	23.39	148.2

As can be seen from Table 2, when vitamin A is deficient rat liver shows increased phosphorus content in the fraction containing nucleoproteins, ribonucleic acid and desoxyribonucleic acid. At the same time vitamin A deficiency is associated with arrest of the animal's growth and it would have been difficult to expect increased protein synthesis under these circumstances. To elucidate this question experiments were performed to determine the rate of synthesis of serum albumin by chick liver slices. The experiments were carried out on liver slices using the immunochemical method as used by I. S. Severina [5].

TABLE 2

Total Phosphorus (in mg %) in Fraction Containing Ribonucleic Acid, Desoxyribonucleic Acid and Nucleoproteins in Liver of Animals with A-Avitaminosis and Control Animals

No.	Total P	
	Experiment	Control
1	210.4	176
2	204.0	169
3	203.0	163.2
4	222.3	163.6
5	221.0	188
6	196.8	173
7	203 3	182.9
8	—	126.7
9	—	166
Average	208.6	167.6

The data obtained showed that in chicks deprived of vitamin A synthesis of serum albumin was practically absent whereas in the control animals which were given vitamin A such synthesis was sufficiently well marked (Table 3).

TABLE 3

Synthesis of Serum Albumin by Liver Slices from Chicks with A-Avitaminosis and Control Chicks During Three Hours' Incubation (in μ g per 1 g Tissue)

No.	Control			Experiment		
	Serum albumin content			Serum albumin content		
	initial	after incubation	increment	initial	after incubation	increment
1	525	740	215	200	240	40
2	240	390	150	540	540	0
3	460	800	340	570	600	30
4	230	430	200	420	420	0
5	910	1200	290	740	750	10
6	900	1065	255	210	210	0
7	560	700	140	430	430	0

It would be premature to correlate the data presented here concerning the increase in nucleic acid content of rat liver and depression of albumin synthesis in chick liver under conditions of A-avitaminosis because of the heterogeneity of experimental material.

Further work on more detailed elucidation of these questions is at present in progress.

SUMMARY

The present work was performed on rats with the aim of clearing up the question of the influence of vitamin A on the contents of nucleic acid.

Lack of vitamin A causes increase of content of ribonucleic acid, as well as of desoxyribonucleic acid.

The second task of this work was to study on chickens the influence of vitamin A deficiency upon the spleen of synthesis of serum albumin.

Under the conditions of vitamin A deficiency the synthesis of serum albumin is negligible.

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THE PRESENCE AND DISTRIBUTION OF ACID PHOSPHATASE IN THE ORGAN OF CORTI IN ANIMALS IN A STATE OF RELATIVE REST AND UPON AUDITORY STIMULATION

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It was established in previous work that under the influence of auditory stimulation a series of cyto-physiologic changes was undergone by the organ of Corti hair cells; these changes characterized the transition of the cells into a state of excitation. Then glycogen which must be regarded as the endogenous energy substrate of the organ of Corti was found in these elements [1]. Finally, the distribution of alkaline phosphatase in the organ was investigated, since it would most probably participate in the maintenance of balance both of glycogen and nucleoproteins [2, 7].

It has been suggested that unlike the tissue distribution of alkaline phosphatase (A.P.), the distribution of acid phosphatase (Ac. P.) is localized mainly in the body of the cell, i.e., that acid phosphatase is directly associated with cell metabolism only. Lately, since the discovery of considerable Ac. P. activity in vegetative neurones, histochemical methods revealing its presence have been recommended as replacement of silver impregnation technics [3, 6]. It is thought that Ac. P. characterizes different functional states of neurones and their peripheral endings [3, 4, 6].

Results of histochemical examination of the organ of Corti for Ac. P. are reported in the present communication.

EXPERIMENTAL METHODS

Acid phosphatase was demonstrated by the Gomori method [5] using different incubation periods. Thirty organs of Corti were studied in serial cross section preparations from 18 cats (10 kittens and 8 adult cats), 6 rabbits and 6 guinea pigs. In the first, control series of experiments 10 animals were maintained in a state of relative rest before being sacrificed by decapitation. In the second series of experiments 10 animals were subjected to one hour of auditory stimulation by high frequency stimuli (1500 cps, 95 decibels) prior to decapitation. In the third series of experiments 10 animals were subjected to low frequency (300 cps, 95 decibels) auditory stimulation for one hour prior to decapitation.

EXPERIMENTAL RESULTS

In the control series of experiments the serial cross sections of the organ of Corti showed reddish-brown passing into black, precipitate (of orthophosphoric acid plumbate) corresponding to the distribution of Ac. P. The enzyme was present in low concentration – light brown precipitate – on the superior convolution of the

organ. Its concentration increased gradually on the middle convolution and reached a maximum on the inferior convolution where the precipitate was black in color. Acid phosphatase was thus distributed along a spiral gradient, its concentration increasing from the superior convolution toward the inferior one. In this respect the distribution gradient for Ac. P. in the organ of Corti proved to be a mirror image of the gradient found by us for alkaline phosphatase. The gradient included all the structural elements of the convolution. There is no doubt that the presence of a distribution gradient reflects functional differences between the elements of the organ of Corti depending on the position of these elements at the level of one or other of the cochlear convolutions. The part of the Reissner membrane facing the endolymph was lined with squamous epithelium and acid phosphatase was only found in the nuclei of the latter. The enzyme was concentrated on the nuclear membrane in karyoplasm aggregates and particularly in the nucleoli (Fig. 1). No acid phosphatase was detected in other parts of the Reissner membrane. The enzyme was also totally lacking from the structural elements of the basilar membrane, the tectorial membrane and the Kelliker membrane.

Fig. 1. Distribution of acid phosphatase in the external hair cells of Corti's organ at the level of the inferior (third) convolution of a young rabbit subjected to sound stimulation (1500 cps, 95 decibels) for 1 hour. Oil immersion objective. Cross section preparation. Microphoto.

Acid phosphatase was traced to the inner sensory hair cells where it was found in the cytoplasm, the nucleus and in the hairs. It was found in low concentration in the phalanges and phalangeal cells. The enzyme can be demonstrated most clearly in the outer hair cells in which it is concentrated in the vacuolated cytoplasm and in the nucleus. The nuclear membrane is sharply delineated. The karyoplasm consisted of nonuniformly colored aggregates and 1-2 outlined nucloli. The sensory hairs in the outer hair cells showed considerable concentration of acid phosphatase.

It should be pointed out that concentration of acid phosphatase increased spirally not only from the superior convolution toward the inferior one, but sometimes also in the radial direction – from the inner hair cells toward the outer hair cells. This radial gradient, however, was not constant. At times other areas were found within the limits of the same convolution in which acid phosphatase concentration increased in the region of the inner hair cells. No acid phosphatase was found in the pillar cells, Deiters, Hensen and Claudius cells; nor was it detected in the vascular strip. Likewise no acid phosphatase was found in the structures enumerated above either in the second or third series of experiments.

Sometimes the presence of acid phosphatase could be detected in some neurones of the spiral ganglion adjacent to one or other convolution and also in nerve fibers passing toward the organ of Corti. It was concentrated in the cytoplasm and the nucleus showing marked preponderance to the area of the nucleolus and at the site of origin of the peripheral process. Acid phosphatase was absent from satellites, blood vessels and connective tissue of the ganglion.

In the second series of experiments, using sounds of high frequency (1500 cps), the distribution gradient for acid phosphatase was in general similar to that established in the control series. Concentration of the enzyme remained practically unaltered in the superior convolution while in the inferior and to some extent in the middle convolutions it was somewhat diminished; the spiral gradient was nonetheless maintained. The cytoplasm of the hair cells of Corti's organ, both inner and outer, became more transparent in the inferior and to some extent in the middle convolutions. The size of the nuclei was changed: some of the nuclei were swollen and markedly enlarged whereas others diminished and shrunk. The outline of the nuclear membrane became pale. The karyoplasm became homogeneous and the nucleoli became paler. The sensory hairs continued to preserve a fairly high concentration of acid phosphatase. The angle formed by the hairs acquired an elliptical shape owing to contraction of the body of the outer hair cells which assumed a more spherical shape, these changes being characteristic for a state of excitation. Changes of acid phosphatase concentration in the phalangeal cells must be pointed out; they often showed alternation of pale areas with darker ones.

Fig. 2. Acid phosphatase distribution in the nuerones of the spiral (eighth) ganglion at the level of the superior (first) convolution in a young rabbit subjected to sound stimulation (300 cps, 95 decibels) for 1 hour. Immersion magnification. Total, cross section preparation. Microphoto.

In the neurones of the spiral ganglion of the inferior and partly of the middle convolutions concentration of acid phosphatase in the cytoplasm, the nucleus nucleoli and dendrites of the nerve cells could often be observed. In the more translucent zones of Corti's organ it was sometimes possible to trace separate fine fibers intertwining with one another in the area at the base of the outer and inner hair cells. These fibers with definite concentration of acid phosphatase represent parts of the outer and inner spiral plexus of Corti's organ. They apparently emerge in connection with functional loading of the corresponding parts of the cochlea.

In the third series of experiments in which sounds of low frequency (300 cps) were employed the spiral gradient was also, on the whole, preserved, as was also the radial gradient (in parts). But in this case there was

a sharp fall in the concentration of acid phosphatase in the region of the hair cells of the superior convolution, while it remained practically unchanged in the middle and superior convolutions. The fall in acid phosphatase concentration in the helicotrema region was so marked that it was almost entirely absent from the hair cell cytoplasm and was barely discernible in their nuclei and particularly the nucleoli. The nuclei were in some cases enlarged, in others diminished. The acid phosphatase concentration also decreased in the hairs, phalanges and phalangeal cells with consequent transparency of the tunnel. It should, however, be mentioned that within the limits of the first convolution, on its descending part, there was already noticeable increase in acid phosphatase concentration.

Changes in the spiral ganglion at the level of the superior convolution proved to be of particular interest. In association with the general fall in concentration it was possible to trace successfully numerous bipolar neurones containing acid phosphatase. Concentration of the enzyme in individual neurones was seen to be unequal. In some its content was extremely high, particularly in that part of the cytoplasm from which the dendrite comes off; in this case the neuronal nucleus was well outlined by its membrane and the nucleolus was well traced (Fig. 2). In other neurones the concentration of acid phosphatase was lower, although it was present both in the cell body and in the nucleus and nucleolus. In the neurones with high acid phosphatase concentration a peripheral process was clearly visible which sprouted from that pole of the cell which was distinguished by high concentration of the enzyme. The axon was at the opposite pole and was more transparent. Bundles of fibers with increased acid phosphatase content were found between the neurones and the processes coming off from their cell bodies; these fibers evidently originated from other parts of the cochlea or, perhaps, represented the end branching of the vegetative bundle of Rasmusen (Fig. 3).

Fig. 3. Acid phosphatase distribution in the nerve fibers of the inner and outer spiral plexus of Corti's organ at the level of the superior (first) convolution in the helicotrema area of a young rabbit subjected to sound stimulation (300 cps, 95 decibels) for 1 hour. Immersion magnification. Cross section preparation. Microphoto.

It proved possible to trace successfully the further course and endings of the spiral neurone dendrites in the region of the superior convolution. Leaving the ganglion they formed a characteristic, widening radial bundle in the region of the spiral lamina; the fibers of this bundle pierced the habenula perforata and passed toward the inner and outer cells of Corti's organ. In the helicotrema region it was possible to note a fairly

large bundle which skirted the edge of the inner hair cells and passing directly under the outer hair cells at their base followed the spiral downward along the convolution. The radial fibers mentioned above, which approached the inner hair cells, formed under their base a very definitely translucent, fairly dense inner spiral plexus. It proved possible to trace the way in which separate fibers of this plexus ended in flask-shaped swellings at the base of the outer hair cells. Part of the fibers from the inner spiral plexus bridged the tunnel and passed under the base of the outer hair cells; here the fibers bent downward at an angle or, forming T-shaped branches, joined those fibers, mentioned above, which had entered in the helicotrema region and formed together the outer spiral plexus. In the latter, three bundles can be clearly discerned which correspond to three rows of outer hair cells as well as individual terminal enlargements which can embrace several receptor elements simultaneously.

The differences in acid phosphatase concentration in the region of the superior convolution evoked by functional "loading" thus permit exceptionally clear demonstration of a number of distinctive structural features of Corti's organ, inaccessible to the usual histologic methods of investigation. Differences in acid phosphatase content of the hair cells in Corti's organ on the one hand, and in the nerve fibers and cell bodies of the spiral ganglion neurones on the other can, in our view, be explained as follows. In the receptor elements of Corti's organ the acid phosphatase is completely used up in catalytic enzymatic processes in connection with increased work. Therefore, its absence may be linked with local prolonged excitation of receptor elements. In the neurones and fibers of the spiral ganglion on the other hand excitation spreads by way of alternating impulses. During the "intervals" between these some part of the enzyme can, apparently, undergo restoration.

Consequently, the results obtained from studies on cochlear convolutions, receptor cells and neurones of Corti's organ permit consideration of differences in acid phosphatase concentration in the structural elements studied as an indicator of different functional significance.

SUMMARY

A spiral gradient of the distribution of acid phosphatase was discovered in the structural elements of the organ of Corti in animals which were in condition of relative rest.

Low concentration of the enzyme was found in the superior (first) convolution of the cochlea.

Its quantity gradually increases in the medial (second) convolution while the highest concentration is revealed in the inferior (third) convolution of the cochlea. Stimulation of animals by sounds of high frequency (1500 cps, 95 decibels) for one hour causes a decrease of the concentration of acid phosphatase in the hair cells of the inferior convolution of the cochlea. Stimulation by sounds of low frequency (300 cps , 95 decibels) on the contrary, causes a decrease in concentration of the acid phosphatase in the cells of the superior convolution. However, stimulation by sound makes the concentration of acid phosphatase more elective in certain neurones of the eighth ganglion, as well as in the nerve fibers of the internal and external spiral network of the organ of Corti.

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PHARMACOLOGY

THE MECHANISM OF BRADYCARDIA INDUCED BY VERATRINE ALKALOIDS

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According to modern concepts, bradycardia induced by veratrine alkaloids [5, 6] as well as by cardiac glucosides [4] is based on a Bezold-Jarisch type of reflex. One of the arguments in support of this point of view is the fact that no such bradycardia occurs upon section of the cervical vagi. Recently, however, we have obtained data indicating that bradycardia induced by veratrine alkaloids could also develop on the basis of a local reflex [1].

It is well known that veratrine alkaloids elicit bradycardia not only of the heart in situ but also of the isolated heart. In connection with this it could be expected that bradycardia arises by a local reflex in the latter case. The present work is concerned with verification of this hypothesis.

The morphologic substrate for local reflexes in the heart has long been established. As far back as the last century A. Dogiel [3] discovered the so-called cells of the second type in the cardiac ganglia, which are regarded by many modern histologists as afferent or sensory neurones of the autonomic nervous system [2]. The

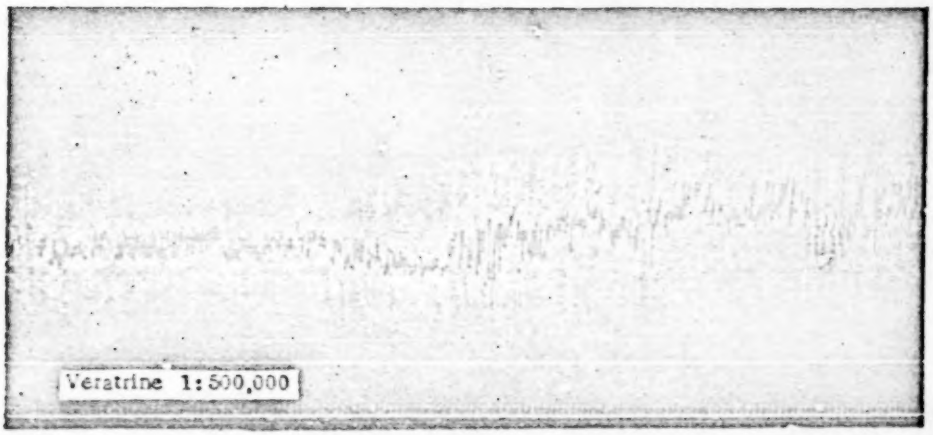

Fig. 1. The effect of veratrine alkaloids on the contractions of an isolated cat heart. Records from above down: cardiac contraction, mark indicating administration of the preparation, time marker (1 second).

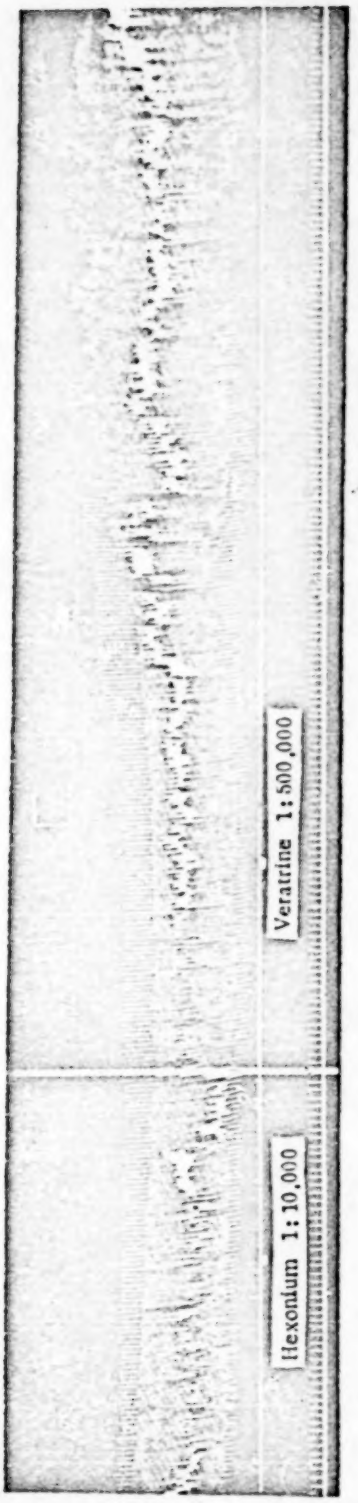

Fig. 2. The effect of veratrine alkaloids on contractions of the isolated cat heart following its treatment with hexonium.* Records the same as in Fig. 1.

efferent pathways of local reflexes in the heart are mediated by the postganglionic neurones of the vagi. Thus, upon stimulation of receptor formations in the heart the transmission of impulses from the afferent pathways to the efferent may be achieved in the cardiac ganglia.

METHODS AND RESULTS

Our experiments were for the most part performed on isolated hearts. The majority of experiments was carried out on cat hearts, the rest on white rat hearts. The hearts were isolated by the Langendorf method and were perfused with Kravkov's fluid, using the usual apparatus. The veratrine alkaloids were used in the form of a mixture – a Merck preparation called Veratrinum purissimum.

In the first series of experiments a check was made on the influence of veratrine alkaloids on the rhythm of contractions in the isolated cat heart. As should have been expected, these alkaloids produced definite bradycardia in relatively low concentrations ($1 \cdot 10^{-6}$) (Fig. 1) and arrhythmia and cardiac arrest in high concentrations ($1 \cdot 10^{-5}$ and higher).

In the next series of experiments the effect of veratrine alkaloids on the rhythm of cardiac contractions under the influence of ganglion-blocking substances was investigated, bearing in mind that if bradycardia so induced arose as the result of local reflexes it could be prevented by blocking transmission of impulses in the cardiac ganglia.

The ganglion-blocking substances used included pentamin** ($1 \cdot 10^{-5}$), hexonium** ($1 \cdot 10^{-4}$), novocaine ($1 \cdot 10^{-5}$) and barbamyl ($4 \cdot 10^{-5}$). It was found that these substances, with the exception of hexonium were not entirely suitable for this purpose since simultaneously with blocking ganglionic transmission in the heart they depressed cardiac activity; in other words they themselves caused slowing of cardiac contractions. Hexonium, however, proved a most convenient preparation for this investigation since it blocked transmission of impulses in the ganglia without causing appreciable depression of cardiac contractions. Following preliminary perfusion of the cardiac vessels with a solution of hexonium bradycardia was elicited by veratrine alkaloids to a smaller extent and in some cases no bradycardia at all was observed (Fig. 2).

Cardiac arrest under the influence of veratrine alkaloids occurs much later following treatment with ganglion blocking substances. For example, when under ordinary conditions veratrine alkaloids led to

* Transliterated. Probably hexamethonium – Publisher.
** Russian trade name.

arrest of the isolated rat heart in concentration of $1 \cdot 10^{-5}$ 3-6 minutes after its administration, following treatment of the heart with hexonium such effect was only achieved after 6-12 minutes.

It was important to establish the effect of veratrine alkaloids on cardiac contractions under conditions of experimental myocarditis since it has been shown that in the latter condition, produced by adrenalin and theophylline, transmission of impulses in the cardiac ganglia of the vagi is either hindered or completely blocked.

Myocarditis was induced in cats by successive intravenous injection of adrenalin (0.3-0.35 ml/kg), 0.1% solution of the hydrochloride, and theophylline (75 mg/kg). The onset and development of myocarditis was monitored by electrocardiograms and by the state of excitation transmission from the vagi to the heart. When the presence of myocarditis was established objectively, the heart was excised and placed in the usual apparatus for perfusion of isolated organs.

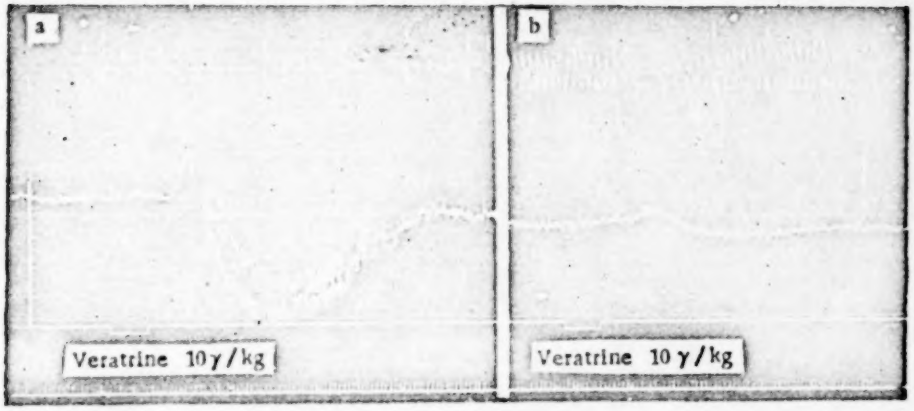

Fig. 3. The effect of veratrine alkaloids on blood pressure before (a) and after (b) introduction of hexonium into the blood.
Records from above down: blood pressure, administration of veratrine, time marker (1 second).

The results of these experiments corresponded to those of the preceding ones: veratrine alkaloids caused less marked bradycardia or no bradycardia at all when transmission of impulses in the cardiac ganglia was either prevented by ganglion blocking substances or hindered by conditions associated with experimentally produced myocarditis. In the latter case cardiac arrest by veratrine alkaloids occurred after longer delay.

The effect of veratrine alkaloids on the rhythm of cardiac contractions following the use of ganglion blocking substances was also studied in intact animals. In these cases, too, bradycardia was less pronounced (Fig. 3).

Finally, the effect of veratrine alkaloids on the rhythm of cardiac contractions in the presence of experimentally produced myocarditis was checked in experiments on intact animals.

The results of these experiments also coincided with the data obtained in experiments on the isolated heart, i.e., in the presence of experimental myocarditis bradycardia caused by veratrine alkaloids (10-20 mg/kg) was considerably less marked than in the normal heart.

Bradycardia of the isolated heart caused by veratrine alkaloids can thus be diminished by hexonium and other ganglion blocking substances. Moreover, these alkaloids give less pronounced slowing of cardiac contractions in the presence of experimentally produced myocarditis in the isolated heart, when transmission of impulses in the cardiac ganglia of the vagi is hindered. There are, therefore, grounds for considering that bradycardia of the isolated heart caused by veratrine alkaloids in relatively small doses develops along the line of local reflexes.

The suggestion that excitation of the vagal ganglia in the heart occurs under the influence of these alkaloids is refuted by the fact that the transmission of impulses from the vagi to the heart is not facilitated but inhibited in these cases.

In the intact organism, no veratrine alkaloid-induced bradycardia is observed after transection of the cervical vagi. Consequently, in the intact organism bradycardia arises reflexly by the Bezold-Jarisch type of effect. The question regarding the reason why local reflexes in the heart do not appear under the influence of veratrine alkaloids so far remains unanswered. It can only be supposed that only with complete denervation of the heart is it possible for local reflexes to arise in it.

Analogous effects may be observed under the influence of cardiac glucosides.

It follows that local reflexes can arise in the heart not only under the influence of veratrine alkaloids but also of cardiac glucosides. Consequently, this phenomenon is of a general character.

SUMMARY

Veratrine alkaloids in small doses cause bradycardia. This action depends mostly on the Bezold reflex. Under the effect of veratrine alkaloids bradycardia may appear in the isolated heart of the local reflex type. It is not yet clear why local reflexes of the heart in situ do not manifest themselves following section of the vagus nerves.

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THE EFFECT OF SOME GANGLION-BLOCKING SUBSTANCES ON THE CORONARY CIRCULATION

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Substances which block transmission of excitation in autonomic ganglia are now widely used for the treatment of hypertensive disease and disorders of peripheral circulation. In view of the fact that treatment of hypertensive disease is complicated, in most cases, by manifestations of angina pectoris it is important that in the clinical use of hypotensive substances their influence on blood supply of the heart be taken into due consideration.

At the same time the abundant literature dealing with the pharmacology of ganglion-blocking substances lacks detailed data on the influence of these preparations on coronary circulation.

Investigations devoted to the study of ganglion-blocking agents and their action on the cardio-vascular system are mainly concerned with their hypotensive effect. References made in some papers to the effect of tetrammonium and hexonium on the blood supply of the heart [7, 12] do not give a sufficiently full idea of the action of these substances. A few papers contain information about the influence of the substances mentioned on cardiac activity, minute volume and circulation in certain vascular areas [3, 4, 5, 8, 11]. Similarly few and contradictory are data on the results of using ganglion blocking agents in angina pectoris [1, 2, 6].

The aim of the present work was to study the effect of a series of ganglion-blocking substances on the coronary circulation. Four preparations were selected for investigation: tetrammonium, hexonium, pentamine and a new ganglion blocking substance – mecamine.*

EXPERIMENTAL METHODS

Experiments were performed on cats under urethane and chloralose anesthesia. The cardiac circulation was judged by the rate of volume output from the coronary sinus.

After opening the chest and instituting artificial respiration a polyethylene catheter was passed through the right auricle into the ostium of the coronary sinus. Coagulation of the blood was prevented by heparin (1000-1500 units/kg intravenously). The polyethylene catheter was connected by a system of rubber tubes with

* Mecamine (corresponding to mevasine) – 3-methylaminoisocamphane hydrochloride – was synthesized at the Institute of Pharmacology and Chemotherapy, AMN SSSR by A. Ya. Khorlin. Detailed pharmacologic studies on this compound were carried out by Yu. V. Uranov (1957).

a special apparatus for recording blood flow. A detailed description of the techniques has been reported earlier [3]. The amount of blood output by the cat coronary sinus varies from animal to animal within the range of 5 to 15 ml/min. Blood pressure was recorded from the carotid artery by means of mercury manometer.

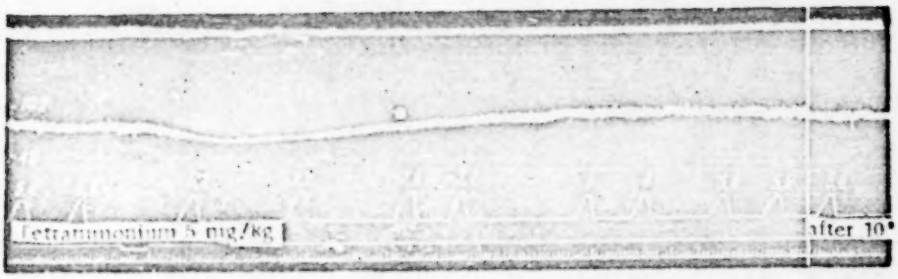

Fig. 1. The effect of tetrammonium on the rate of volume output from the cat coronary sinus. Records from above down: amplitude of cardiac contractions, blood pressure, blood output (in ml/min), administration of the substances, time marker (5 seconds). The height of the deflections corresponds to the rate of volume output of blood over 15 seconds. Figures above the deflections – volume of blood flow per minute.

In a number of experiments the amplitude of cardiac contractions was recorded with the help of a myocardiograph with pneumatic transmission. The preparations under investigation were administered intravenously.

EXPERIMENTAL RESULTS

The experiments showed that tetrammonium in doses of 2-3 mg/kg increased the coronary sinus blood output by 8-10% as compared with the initial level. It increased the output by 25-30% in cases where the initial values of the rate of blood flow were low (5-6 ml/min). Increasing the doses of tetrammonium to 5-10 mg/kg led to an increase of output by 40-60% in relation to the initial level (Fig. 1).

Tetrammonium thus possesses the ability to increase the rate of coronary blood flow. Its effect increases with increasing doses. However, depending on the initial state of blood supply to the heart, analogous doses of tetrammonium may elicit different effects. The increase in blood supply to the heart caused by tetrammonium lasts 10-15 minutes. Even when 10 mg/kg tetrammonium is given the coronary sinus blood output returns to the initial value after 15 minutes.

Administration of tetrammonium was accompanied by falls of blood pressure to different degrees in different animals.

The average fall in blood pressure following administration of 2-5 mg/kg tetrammonium was 15-20 mm Hg and persisted for 12-15 minutes.

It must be noted that tetrammonium decreased the amplitude of cardiac contractions.

If the action of tetrammonium on coronary circulation depends in each case on the state of the blood supply to the heart, then it is even more true of hexonium. As shown by the experiments, in cases in which the values of blood output were medium (7-8 ml/min) hexonium in doses of 2-3 mg/kg increased the rate of blood flow by 25-40% as compared with the initial level. A fall in blood pressure was noted at the same time; the average drop was 20-25 mm Hg and there was a slight diminution in the amplitude of cardiac contractions. In animals with a more vigorous blood supply to the heart muscle (minute blood flow volume – 10-15 ml/min) analogous doses of hexonium led to marked (20-40%) decrease of blood flow rate (Fig. 2). Such dependence of the reaction and its degree is also characteristic for the action of large doses of hexonium (5-7 mg/kg).

Pentamine in doses of 2-3 mg/kg decreased blood output from the coronary sinus. In the presence of adequate blood supply to the heart (output value 10-12 ml/min) the decrease of volume output per minute under the influence of pentamine constituted 40-50% while in cases of lower blood supply (5-6 ml/min) it constituted 15-20%.

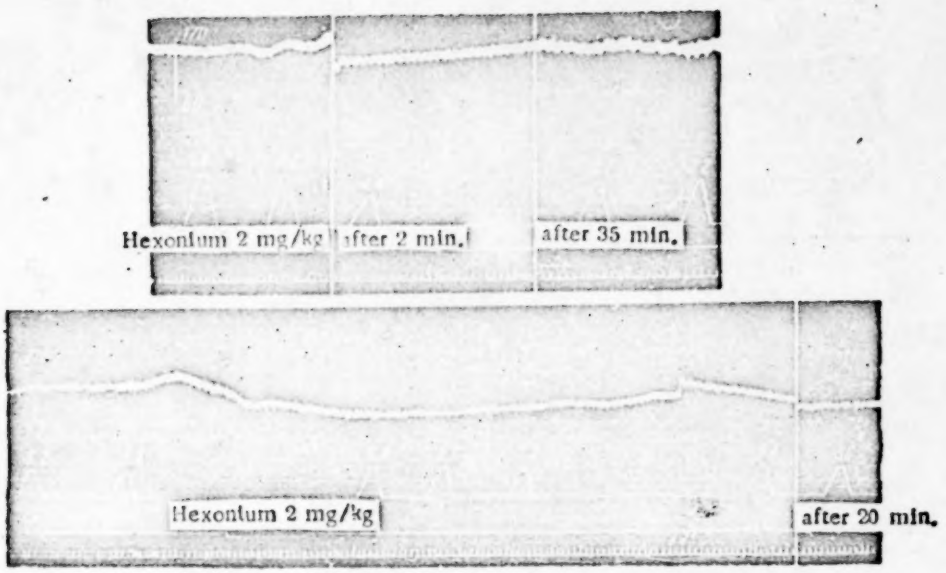

Fig. 2. The effect of hexonium on the rate of volume output from the cat coronary sinus. Top – high values of initial coronary volume output per minute; bottom – low values of initial coronary volume output per minute. Records from above down: blood pressure, blood output (in ml/min), administration of substance, time marker (5 seconds).

Pentamine in doses of 5-7 mg/kg led to less definite decrease of minute blood flow and in some cases, in which the blood supply to the myocardium was lowered, caused its slight increase (by 12-15%).

Administration of pentamine was seen to be accompanied by considerable (20-25 mm Hg) lowering of blood pressure and diminution of amplitude of cardiac contractions.

Changes in the minute coronary blood flow caused by pentamine were observed during 20-30 minutes after which cardiac blood supply became stabilized at the initial level (Fig. 3).

Mecamine, like pentamine, usually decreased coronary circulation. In doses as low as 0.5-1 mg/kg mecamine lowered the rate of blood flow by 20-50% compared to the initial level. The action of mecamine on coronary circulation lasted 20-30 minutes. Variations in the changes elicited by mecamine were connected with different states of cardiac blood supply in different animals. In cases where the initial values of minute blood volume were low (5-6 ml/min) the latter was slightly (by 12-15%) decreased by mecamine or, as occurred in a number of cases, no effect on coronary circulation was observed. Unlike the other ganglion-blocking agents investigated, mecamine, even in large doses (3-5 mg/kg) never led to increased blood output from the coronary sinus. Administration of mecamine (1-2 mg/kg) was accompanied by a gradually developing, prolonged lowering of blood pressure amounting, on the average, to 20-30 mm Hg.

Mecamine caused some diminution in the amplitude of cardiac contractions.

The results of these investigations permit the conclusion that changes in coronary circulation elicited by ganglion-blocking substances vary within wide limits.

Variation in the reactions of the vascular system of the heart in response to the administration of ganglion-blocking agents depends in each individual case on the state of blood supply. The value of the initial minute coronary blood flow in many cases determines not only the degree but also the sign of the reaction.

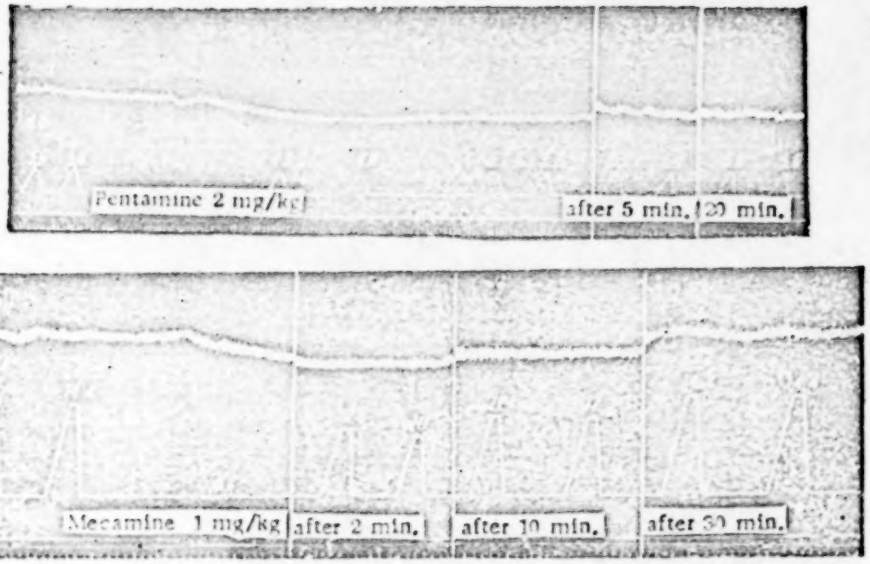

Fig. 3. The effect of pentamine (top figure) and of mecamine (lower figure) on the rate of volume output from cat coronary sinus.
Records as in Fig. 1.

Experimental and clinical observations show that the hypotensive action of ganglion-blocking substances is not constant. The intensity of hypotension elicited by them depends on the initial tonus of the vasoconstricting nerves [9, 10]. Our observations indicate that this is evidently also significant for the vascular system of the myocardium.

Among the ganglion-blocking substances investigated tetrammonium gave the most constant increase of coronary circulation. Increase of the minute coronary blood flow is observed upon administration of hexonium if the blood supply to the heart is initially low. Pentamine and mecamine decrease the rate of coronary blood flow in most cases.

The experimental data presented should be taken into account when selecting ganglion-blocking substances for the treatment of hypertensive disease accompanied by impairment of coronary circulation.

SUMMARY

Acute experiments were performed on cats. The study of the effect of ganglioblockers (tetrammonium, hexonium, pentamine and mecamine) on the blood flow from the coronary sinus was carried out.

The effect of these substances on the coronary circulation is variable and depends on the initial conditions of the blood supply of the heart.

Tetrammonium in the dose of 2-10 mg per kg of body weight increases the blood flow from the coronary sinus by 10-40%. Hexonium (2-5 mg per kilogram) increases the blood flow from the coronary sinus by 25-40% in case of the average and low, initial values of the volume of the blood flow per minute. Hexonium in the above dose causes decrease of the volume velocity of the coronary blood flow by 20-40% in animals with intensive myocardial blood supply.

In majority of cases pentamine in the dose of 2-5 mg per kilogram of body weight and mecamine in the dose of 0.5-2 mg per kilogram cause decreased blood flow from the coronary sinus.

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** In Russian.

THE INFLUENCE OF NOVOCAINE ON PESSIMAL INHIBITION IN VARIOUS LINKS OF THE REFLEX ARC

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Novocaine has in recent years been shown to possess a wide spectrum of action. Besides the generally known properties of anesthesia, novocaine shows the following effects upon resorptive action: depression of transmission of excitation in the central nervous system [2-7], in autonomic ganglia [1, 9, 10, 13] and partially in neuro-muscular synapses [13-16], as well as in the region of m-choline receptors [11].

In the present investigation an attempt was made to discover certain aspects of the mechanism of novocaine action within the framework of studies on its influence on processes of pessimal inhibition in different links of the reflex arc.

The influence of novocaine on neuro-muscular transmission. Experiments were carried out on decerebrate cats. Records of the contractions of the gastrocnemius muscle were made under semi-isometric conditions. The peripheral segment of the tibial nerve was stimulated by square-pulse stimuli each of 0.5 millisecond duration. The frequency of stimulation was varied from 0.5 up to 300 cps. Stimulation at each frequency was continued for 10 seconds. Novocaine was given intravenously.

Novocaine in doses 2.5-5 mg/kg did not exert any appreciable effect on neuro-muscular transmission. The amplitude of the muscular contractions on stimulation at frequencies of 0.5-10-20 and 50 cps increased slightly or remained unchanged when 10-15 mg/kg novocaine had been administered. At the same time a more rapid decline of the tetanic curve at frequencies of 80-200 cps was observed. Primary twitching at these frequencies was somewhat more pronounced than in the normal. In doses of 25 mg/kg novocaine led to a definite decrease in the amplitude of the myogram at all frequencies of stimulation (Fig. 1). The pessimal reaction began to be apparent at 50 cps and became most definite at 80-200 cps. Restoration began fairly rapidly and after 12-20 minutes was already practically complete. The maximal effect of the substance was attained in the first 5 minutes following its injection.

On repeated injection of novocaine its effect was less marked than that achieved after the first injection.

There are references in the literature to the fact that novocaine can diminish acetylcholine production [13]. With this in mind, experiments were carried out in which the effect of proserine* on the activity of novocaine was examined. Proserine was used in doses of 20-40 kg. Under these conditions an increase in the amplitude of single contractions and a considerable shift of the pessimum toward the lower frequencies were observed. The primary twitch of the muscle was somewhat increased with the range of frequencies from 50-200 cps. Administration of novocaine against this background (25 mg/kg) lowered the amplitude of muscular contractions to a considerable extent. Pessimal reaction could already be seen at a frequency of 50 cps.

* Russian trade name.

The action of novocaine passed after 35-40 minutes, whereas changes in neuro-muscular transmission associated with the effect of proserine persisted. This interrelation could also be observed when smaller doses of of both the substances were used. Thus, the synergism of proserine and novocaine was observed when proserine was used in such doses (1-5 γ/kg) which by themselves did not alter neuro-muscular transmission appreciably.

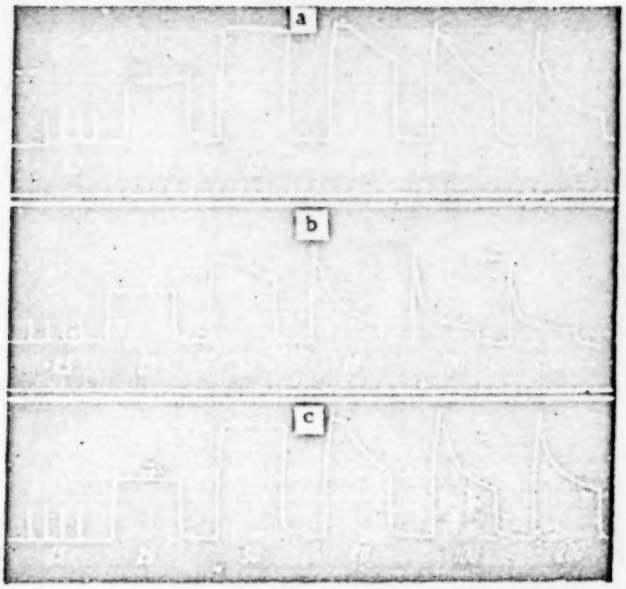

Fig. 1. The effect of novocaine on neuro-muscular transmission. a) Before administration of the substance; b) 2 minutes after injection of 25 mg/kg novocaine; c) after 15 minutes. Frequency of stimulation of the tibial nerve is marked by figures (in cps).

The effect of novocaine on autonomic ganglia.

These experiments were carried out mainly on the superior cervical ganglion of decerebrate cats. Contractions of the nictitating membrane were recorded upon stimulation of the preganglionic trunk by square-pulse stimuli at various frequencies. The technique used has been described earlier [12].

The minimal dose of novocaine which exerted an influence on the level of pessimal inhibition in the ganglion was equal to 1-2 mg/kg. The effect was observed particularly clearly when novocaine was used in doses of 5-10 mg/kg (Fig. 2) and higher. In Fig. 2 the initial pessimal frequency was 200 cps; relaxation of the membrane took place gradually. Following administration of novocaine (10 mg/kg) the pessimum occurred at a frequency of 80 cps and the decline of the curve was very steep. Change of the pessimal frequency to the optimal (10 cps) led to restoration of the curve. Such a dependence of the effect on the frequency indicates that the observed inhibition is pessimal. It is characteristic for novocaine that there is usually a change in the reaction to optimal frequency of stimulation too, following its administration. The same figure shows that the height of the mechanogram is decreased at the frequency of 10 cps. This means that the excitability of the ganglion cells is lowered under the influence of novocaine. The possibility is not excluded that a definite role may be played by a reduction of acetylcholine production [13]. Doses of 5-10 mg/kg and higher are associated with a shift of the pessimum toward lower frequencies which occurs parallelly with diminution of the excitability of the ganglion cells. It is possible to observe changes elicited by pessimal inhibition without appreciable lowering of ganglion cell excitability by using novocaine in threshold and near-threshold doses.

The duration of novocaine action is not long. It is usually of the order of 20-30 minutes.

Confirmation of the ganglionic action of novocaine was obtained in experiments with recording of biopotentials from the postganglionic fibers. The preganglionic sympathetic trunk was stimulated at various frequencies. It was shown in these experiments that novocaine decreased the amplitude of the biopotentials and shortened the time during which they could be reproduced.

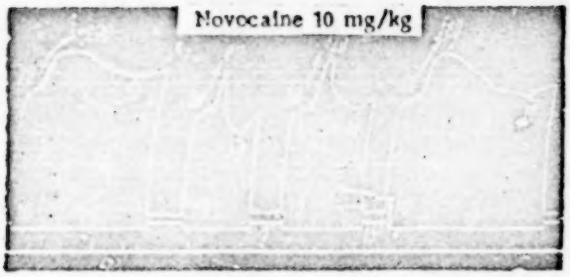

Fig. 2. The effect of novocaine on the lability of the superior cervical ganglion.
Figures indicate the frequency of stimulation of the preganglionic trunk in cps. The gradual transition from lower to higher frequencies is marked by arrows; time marker (30 seconds).

The effect of proserine on the action of novocaine was also studied experimentally in a way similar to that used in the experiments on neuro-muscular transmission. Proserine was used in doses of 20-50 γ/kg and higher. There was no noticeable change in ganglionic transmission of impulses when proserine alone was used. The action of novocaine against the background of proserine (20-30 γ/kg) was somewhat enhanced. Duration of the effect was practically unaltered.

Effect of novocaine on spinal reflexes.

The experiments were performed on lumbar cats. The spinal cord was transected at the level of the lower thoracic vertebrae. Reflex contractions of the semitendinosus muscle were recorded under semi-isometric conditions. The central segment of the fibular nerve (of the same limb) was stimulated by square-pulse stimuli with a duration of 0.1-0.5 millisecond. Stimulation at each frequency was continued for 10 seconds. The intervals between these were no less than 25 seconds, the intervals between each series being 7-10 minutes. Frequencies from 0.5 to 100 cps were used. Novocaine was given intravenously.

Novocaine in doses of 5-10 mg/kg and higher lowered the amplitude of muscular contractions at all frequencies of stimulation of the nerve trunk. Particularly definite changes occurred at the frequency of 0.5 cps. In this case administration of novocaine (12.5-25 mg/kg) was followed by decrease in the height of the mechanogram accompanied by a more rapid relaxation of the muscle. The character of the pessimal reaction, observed as a decline in the tetanic curve, did not change at higher frequencies of stimulation which was evidently connected with enhanced change in the central link of the reflex arc. As recovery from the action of novocaine proceeded there was some facilitation (as compared with the initial) of the reflex transmission of excitation.

Repeated injection of novocaine produced a less marked effect.

In order to investigate the influence of novocaine on pessimal inhibition in the spinal cord, a series of experiments was carried out in which biopotentials were recorded from the deep (motor) branch of the tibial nerve. The more superficial (sensory) part of the same nerve was stimulated by square-pulse stimuli of various frequencies and duration. In these experiments novocaine reduced the amplitude of the potentials. The ability of the cord to reproduce biopotentials over a certain period of time was altered, with shortening of this

period of time. Transformation of the rhythm after administration of novocaine occurred considerably sooner and was more pronounced than before administration of the substance (Fig. 3).

The data obtained indicate that novocaine favors the development of pessimal inhibition in the reflex centers of the spinal cord, sympathetic ganglia and neuro-muscular junctions. Sympathetic ganglia and spinal cord (lumbar segments) are more sensitive to novocaine than neuro-muscular junctions.

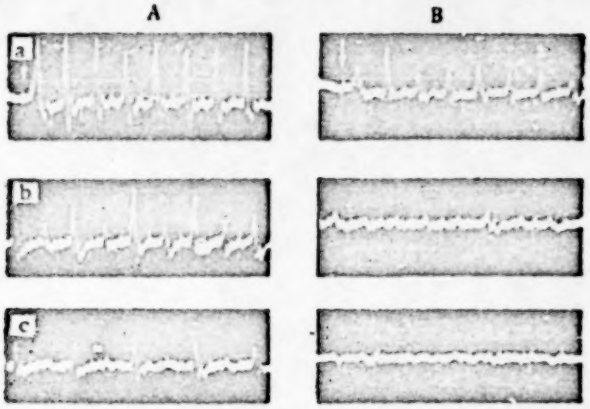

Fig. 3. Effect of novocaine on the lability of spinal centers. Biopotentials before (A) and after (B) administration of 15 mg/kg novocaine.
a) Beginning of stimulation (shown by arrow); b) 5th second after beginning of stimulation; c) 10th second. Biopotentials led off from the motor part of the tibial nerve with electric stimulation of the sensory branch of the same nerve. Frequency of stimulation – 40 cps. Duration of each stimulus – 0.1 millisecond. Short vertical lines – stimulus artifacts.

Attention should be drawn to the fact that when novocaine is combined with proserine the depressing effect of the former with respect to neuro-muscular and ganglionic transmission is not only not weakened but even enhanced to a certain extent. These observations do not agree with the fairly widely held view that the blocking effect of novocaine is caused by its ability to depress acetylcholine synthesis [14 and others]. This view is based on data obtained by Harvey [13] on the superior cervical ganglion. He succeeded in noting that novocaine diminished acetylcholine production connected with stimulation of the preganglionic trunk. However, it was shown in the same work that novocaine diminished the ganglion and skeletal muscle reaction to acetylcholine administered intra-arterially. There is a fairly large number of similar investigations applied to striated muscle [14]. Under such experimental conditions the influence of novocaine on acetylcholine synthesis certainly cannot be the cause of the absence of reaction to acetylcholine. The possibility of alteration in cholinesterase activity is also excluded since in experiments on the superior cervical ganglion the latter was perfused with a solution containing eserine. All this suggests that novocaine exerts a direct influence on synaptic formations also.

Comparison of our data with observations made by Kaverina and Khayutin (1954) draws attention to the considerable difference in the doses of novocaine needed to inhibit the spinal cord. Thus, according to Kaverina and Khayutin, novocaine in doses of 5-7 mg/kg caused considerable inhibition of the flexor reflex in the cat hind limb and blocked it completely in the dose of 16.5 mg/kg. We did not succeed in obtaining similar results. Even in the dose of 25 mg/kg novocaine produced a relatively slight inhibiting effect. A series of control experiments established that such discrepancies were connected with different experimental

conditions. One such difference lay in the fact that our experiments were performed on lumbar cats without any anesthesia while Kaverina and Khayutin tested novocaine on animals under urethane anesthesia (0,5 to 1 g/kg). And urethane is known to inhibit reflex activity of the cord to a significant extent. Against such a background novocaine does indeed block transmission of excitation when given in doses cited by Kaverina and Khayutin. The discrepancy between our data and those reported by Kaverina and Khayutin are thus explained by differences in the functional state of the spinal centers in the experimental animals. As has been shown in the present investigation, considerably smaller doses of novocaine are required to inhibit the reflex activity of the spinal cord in cats under urethane narcosis than in experiments in which no narcosis is used.

SUMMARY

The mechanism of the action of novocaine was studied experimentally. It was demonstrated that in resorptive action of novocaine it increases the pessimal inhibition in the reflex centers of the spinal cord the vegetative ganglia and the neuro -muscular apparatus. Experiments were performed according to the teaching of Vedensky on the functional mobility of the excitable formations.

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PHARMACOLOGY OF PYROGENIC BACTERIAL POLYSACCHARIDES

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(Received March 4, 1957. Presented by Active Member USSR Academy of Medical Sciences, I. V. Davydovsky)

Pyrogenic polysaccharides obtained in a number of foreign laboratories are known to possess many-sided action on a whole series of organs and systems of both the healthy and diseased organism.

It has been shown in papers published by foreign authors that under the influence of "pyromene" (protein-free pyrogenic bacterial preparation, freed from anaphylactic properties) reparative processes undergo changes (Windl and Chambers [5]), vascular permeability is decreased (L. Greene [3]), leucocytosis sets in (D. Hankel, R. Mefferd, J. Loefer [2]), allergic states are abolished (Randolph and Rollins [4]).

There is no doubt that the properties of pyrogenic preparations have not yet been fully discovered. Nonetheless, those properties which are already known present great interest both for experimental and for clinical medicine.

A preparation of a pyrogenic polysaccharide has been obtained in Professor M. V. Svyatukhin's laboratory from a culture of Proteus vulgaris,* and this preparation has been the material used in this work.

EXPERIMENTAL METHODS

The general action and toxicity of the preparation were studied on sexually mature white mice weighing 20 g (± 2 g). The preparation was given intraperitoneally (in solution), starting with 10 γ and up to 5000 γ per 1 kg body weight. Within this range of doses no changes, apart from some mild depression, were noted in the general state and behavior of the animals. Large doses could not be tried owing to the low solubility of the preparation.

Application of the polysaccharide to the mucosa and its subcutaneous and intramuscular injection revealed no locally irritating action.

EXPERIMENTAL RESULTS

The pyrogenic action of the preparation, injected intravenously or intraperitoneally, is observed in rats, rabbits and dogs when doses as small as 1-2 γ per kg are given. Of all the animals studied the least marked pyrogenic effect was observed in rats. Increasing the dose 10, 100 fold and more does not lead to pronounced enhancement of the pyrogenic effect, i.e., no clear correlation is seen between the pyrogenic effect and the dose of the preparation. The rise in temperature began, as a rule, within the first half-hour after injection and the temperature continued to rise in the course of $1\frac{1}{2}$ - $2\frac{1}{2}$ hours. The body temperature then began to decline reaching the initial level by the 5th - 7th hour.

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* The method of preparation is described in the paper by A. A. Bodareva and M. V. Svyatukhin.

It is known that preliminary intravenous injection of novocaine (in rabbits and dogs) leads to delay in the rise of temperature amounting to $1\frac{1}{2}$ to 2 hours and decreases the extent to which it rises (Fig. 1). Analogous effects were seen in rats under urethane anesthesia. Taking into account the available facts, it may be postulated that hyperthermia arising under the influence of pyrogenic polysaccharide is reflex in nature. It would appear that the reflex arising from vascular receptors is completed by way of the higher thermoregulatory centers. Anti-inflammatory properties of the pyrogenic polysaccharide were studied on rabbits using the technique described by I. A. Oivin and K. N. Monakova [1].

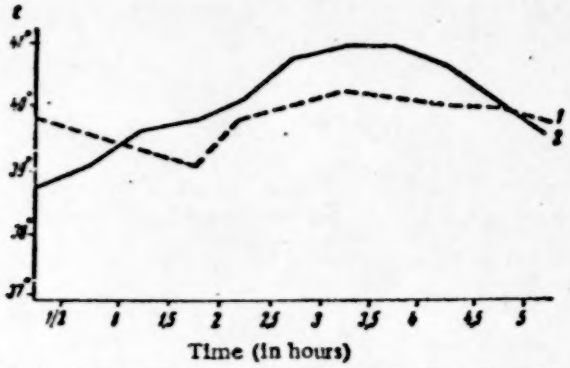

Fig. 1. Temperature reaction in novocaine-treated (1) and untreated (2) dog.

The results of the experiments indicate that under the influence of the preparation a gradual slowing of escape of dye-stuff from the vessels takes place (Fig. 2), i.e., the preparation possesses some slight anti-inflammatory properties.

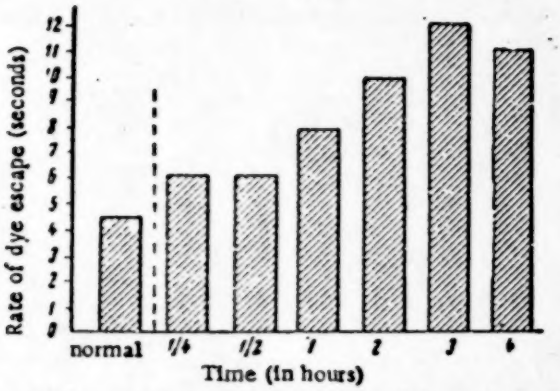

Fig. 2. The rate (in seconds) of escape of dye-stuff from capillaries at different intervals following administration of pyrogenic polysaccharide.

The influence of the bacterial polysaccharide on arterial blood pressure and respiration was studied in acute experiments on nonnarcotized rabbits and dogs under morphine-urethane anesthesia. In these experiments it was found that if intravenous injection of the preparation started with small doses (2-20 γ per kg) then no substantial changes in blood pressure, rate of cardiac contractions and nature of respiration were observed

even if the dose was then sharply increased (500 γ per kg); but if doses of 500 γ per kg and over were given at the very beginning of the experiment a transient fall in blood pressure and depression of respiration down to apnea were observed upon the first injection (Fig. 3). All subsequent injections of the preparation in the same animal do not elicit the same effect even when large doses (1000-2000 γ per kg) are given.

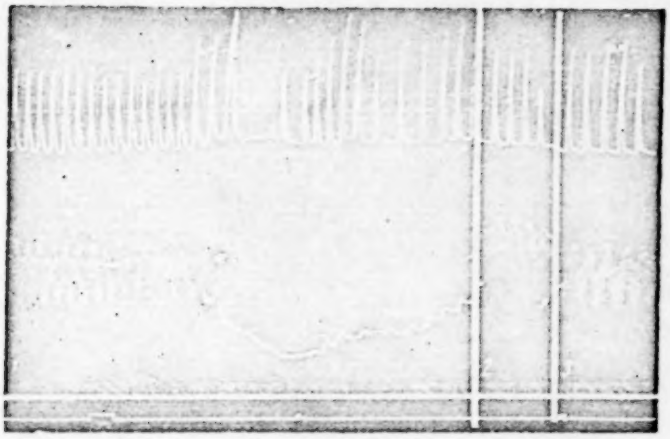

Fig. 3. The effect of a large dose (500 γ per kg) of pyrogenic polysaccharide on arterial blood pressure and respiration upon being injected for the first time.
Records from above down: respiration, blood pressure in the femoral artery, time marker (1 second), injection of preparation. 1) Normal and immediately after injection of preparation; 2) 5 minutes after injection; 3) 10 minutes after injection.

The bacterial polysaccharide in concentrations of 1:50,000 - 1:500 exerts no appreciable effect on the isolated frog heart (Straub method).

The effect on diuresis was studied in experiments on white rats weighing 170-200 g. The preparation was given intraperitoneally and "hydration" (5 ml boiled water per 100 g body weight) was carried out by introducing the water into the stomach. Observations over a period of 5 hours with recording every half hour did not reveal any noticeable difference in diuresis between the control and experimental animals.

The experiments performed may be summarized as follows.

Parenteral administration of the bacterial polysaccharide obtained from Proteus vulgaris cultures elicits a rise of body temperature in rats, rabbits and dogs when given in doses of 1 γ per kg and larger. This rise in temperature continues for 5-7 hours. Preliminary treatment with novocaine or narcotization of the animals produces a delay in the rise of body temperature and diminishes the degree of such rise. Administration of the pyrogenic preparation is associated with decreased permeability of the blood vessels, i.e., the preparation exhibits some anti-inflammatory properties. Small doses of the preparation (2-20 γ per kg) do not affect respiration and blood pressure appreciably; large doses (500 γ per kg and over) elicit transient lowering of blood pressure and depression of respiration. The latter effect occurs only upon the first injection of the preparation. The pyrogenic polysaccharide preparation under investigation is of low toxicity.

SUMMARY

Certain aspects of the pharmacodynamics of bacterial pyrogenic polysaccharides were studied in this work. It was established that there is an increase of the body temperature in animals, which lasts for 6-7 hours, following parenteral administration of this preparation in the dose from 1γ per kilogram and over. Preliminary administration of novocaine and anesthesia to these animals causes delay in the rise of body temperature and decreases the degree of its rise. The preparation has mild anti-inflammatory properties and its toxicity is low. It has no effect on respiration and blood pressure in the doses of 2-20 γ per kilogram of body weight. There is no disturbance of diuresis following administration of this preparation.

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MICROBIOLOGY AND IMMUNITY

A STUDY OF THE NATURE OF THE ALLERGY TO STREPTOCOCCUS IN RHEUMATISM

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(Received May 12, 1957. Presented by Active Member USSR Academy of Medical Sciences, M. S. Vovsy)

Rheumatism is regarded by most authors as an infectious-allergic disease. The prevalent point of view in the current literature is that sensitization in rheumatism is caused by streptococcal sensitizing agents. N. D. Strazhesko considers tonsillitis as a primary cause creating favorable conditions for the development of rheumatism. During tonsillitis, the organism reabsorbs the products of streptococcal growth from the tonsils, causing sensitization against the background of which rheumatism develops. From this, the constant pathogenetic connection between tonsillitis and rheumatism, which is documented by a large number of observations, becomes understandable. According to N. D. Strazhesko, tonsillitis precedes rheumatism in 64% of the cases, according to A. N. Koritsky, V. L. Bisyarina and M. G. Mirkina, in 70-80%, while according to E. N. Nezlin, in an overwhelming majority of patients, given good recollection.

Starting with 1948, evidence has appeared on the importance of blood cells in adsorbing sensitizing substances in various allergic states. A similar fact was initially stated by Chase [4] for tubercular allergy. This author established that in persons who react positively to tuberculin, washed leucocytes from peritoneal exudate sensitize guinea pigs. Following the intracutaneous injection of the latter with tuberculin, they develop a hyperallergic reaction. In 1952, H. Lawrence reported on the sensitization of healthy volunteers who had been injected intracutaneously in the shoulder region with washed leucocytes of persons having a positive reaction to streptococcal allergen. As a result, they developed passive allergy to streptococci, lasting from 1 week to 3 months. On the basis of these experiments, H. Lawrence concluded that leucocytes from people who had earlier suffered streptococcal infections, adsorb substances "resembling tuberculin," i.e., streptococcal sensitizing agents.

The investigations of Chase and Lawrence were the premise for our investigations. We set ourselves the problem of determining whether the leucocytes and erythrocytes of rheumatic patients with positive reactions toward streptococcal allergen adsorb substances of the streptococcal sensitizer type.

EXPERIMENTAL METHODS

Blood was taken from rheumatic patients with strongly expressed allergic reactions to intracutaneous administration of streptococcal allergen and was placed in test tubes containing sodium oxalate (0.01 ml of a saturated solution per 1 ml blood). The contents of the test tube were thoroughly mixed and centrifuged with

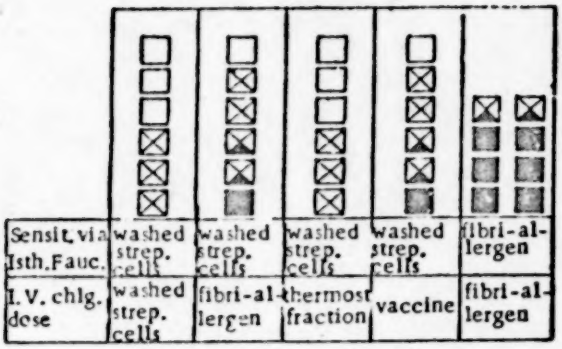

Fig. 3. Results of experiments in which guinea pigs were sensitized with washed microbial cells of the streptococcus in order to determine the presence of sensitizing fractions of the streptococcus in the microbial cells or in the products of its growth, contained in the filtrates of 6-day broth cultures. Designations same as in Figs. 1 and 2.

We set up experiments in which guinea pigs were sensitized with washed cells of a hemolytic streptococcus. For the challenge dose, one group of these animals was injected with washed streptococcal cells, another with streptococcal allergen, a third with the thermostable fraction of allergen prepared by us, and a fourth with streptococcal formol-vaccine. The results of these experiments showed that the use of washed streptococcal cells as sensitizing and challenge doses gave a very weak effect; only half of the guinea pigs showed light anaphylactic manifestations. Only the use of allergen and streptococcal formol-vaccine as the challenge dose caused death in one of each six pigs; two of each group had a severe form of anaphylaxis, and two had a light form of anaphylaxis (Fig. 3).

Such an effect could only be caused by substances from 6-day broth cultures of streptococci which pass through a bacterial filter (F_2). These substances are apparently thermolabile, since the thermostable allergen fraction used as the challenge dose had a weaker activity as did the washed streptococcal cells. When the results of sensitization with washed streptococcal cells and the thermostable allergen fraction are compared with the sensitizing activity of the thermoabile fraction of the allergen, the great sensitizing activity of the latter becomes immediately apparent.

Therefore, in rheumatism it is not the microbial cells of streptococcus themselves that sensitize, but rather the products of their growth, which pass through bacterial filters and are apparently thermolabile. These substances may be either fragments of microbial cells or filtrable forms of the streptococcus.

The facts presented lend new support to the concept of the streptococcal nature of rheumatism. Evidently, allergy to streptococci in rheumatic patients is, as a rule, accompanied by the presence of streptococcal sensitizing agents adsorbed to the formed elements of the blood. Regardless of how we regard these sensitizing agents, either as disintegration products of microbial cells or as filtrable forms, we have a new fact, proving the systematic presence of streptococci in the organism of rheumatic patients. Also, on the basis of O. V. Krasovskaya's work [2, 3], who used the method of staining blood smears of rheumatic patients according to Morozov and electron microscopy there are serious reasons to consider these formations as filtrable forms of

repeated washings with physiological saline and aspiration of the upper layer, containing leucocytes. The sediment, consisting of erythrocytes, was also subjected to washing with physiological saline. As a result, washed leucocytes, on the one hand, and washed erythrocytes, on the other were obtained.

In one set of experiments, we injected guinea pigs with washed leucocytes in the region of the isthmus focium,* in another, washed erythrocytes were injected, while in a third, a mixture of leucocytes and erythrocytes. The sensitizing dose was equal to 0.2-0.4 ml. In 3 weeks, the guinea pigs were injected intravenously with a challange dose of streptococcal allergen (filtrate of 6-day broth cultures of a hemolytic streptococcus). Controls for this investigation were experiments with sensitization of guinea pigs with streptococcal allergen and with the intravenous injection of a challange dose of the latter. In addition, there were animals in the control groups into which broth was injected as the sensitizing and resolving doses, and also washed leucocytes and erythrocytes from the blood of healthy people who did not react to streptococcal allergen. In all, there were 49 guinea pigs in the experimental group and 50 in the control groups.

EXPERIMENTAL RESULTS

Experiments in which 14 guinea pigs were injected with washed leucocytes in the region of the isthmus fociumand subsequently injected intravenously with streptococcal allergen gave rise to anaphylaxis in 7 of the animals. The most interesting experiments were those on 9 guinea pigs, injected with washed leucocytes from Patient D, who had a marked hyperallergic reaction to streptococcal allergen; 4 of the guinea pigs showed typical anaphylaxis, while washed erythrocytes from the same patient evoked mild anaphylactoid symptoms in 2 guinea pigs. However, when leucocytes were used in a mixture with erythrocytes, one of 3 guinea pigs developed severe anaphylaxis, terminating in the death of the animal, while the other 2 guinea pigs showed light signs of anaphylaxis. These experiments showed that leucocytes apparently adsorb streptococcal sensitizing agents more intensively than do erythrocytes in the organism of the rheumatic patient. On the other hand, streptococcal sensitizing agents were adsorbed unequally, since, in some animals, anaphylaxis following sensitization and challenge doses appeared with all its inherent manifestations, while only a weak anaphylactoid reaction was observed in others.

Analogous facts were also recorded when washed leucocytes and erythrocytes from the blood of other patients examined by us were used. In a total of 40 guinea pigs, typical anaphylaxis was produced in 19 animals, while anaphylactoid symptoms were observed in 11 pigs (Fig. 1).

In sensitizing guinea pigs with streptococcal allergen the injection of a challenge dose of the same preparation (filtrate of 6-day broth cultures of streptococcus) evoked anaphylaxis in all of the animals, in most cases terminating in death. In order to determine the specificity of action of the allergen, we added serum from guinea pigs which had recovered from anaphylaxis and which were, according to A. M. Bezredky's determination, in a state of antianaphylaxis. After sensitization of the guinea pigs and the injection of the challenge dose of allergen mixed with serum, anaphylaxis developed in only one of the five animals (Fig. 2). Therefore, sensitization occurs due to a specific streptococcal sensitizing agent against which guinea pigs recover from anaphylaxis produce a neutralizing substance in the period of antianaphylaxis.

Control experiments, with the use of broth alone in the capacity of sensitizing and challenge doses, and also with washed leucocytes and erythrocytes from healthy persons, gave negative results; neither anaphylaxis, nor even anaphylactoid manifestations, were produced in the animals in a single case.

On the basis of the data presented, it is possible to conclude that the leucocytes and erythrocytes in the organism of rheumatic patients adsorb specific streptococcal sensitizing agents which cause allergy in rheumatism.

We were also interested in the question of whether sensitization takes place due to whole microbial cells, or due to their products of disintegration (and possibly filtrable forms) contained in streptococcal culture filtrates.

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* On the basis of our work published in the Byull. Ekspti. Biol. i Med. No. 12, p. 48 (1954), it was established that the region of the pharyngeal arches in guinea pigs is the most sensitive to sensitization.

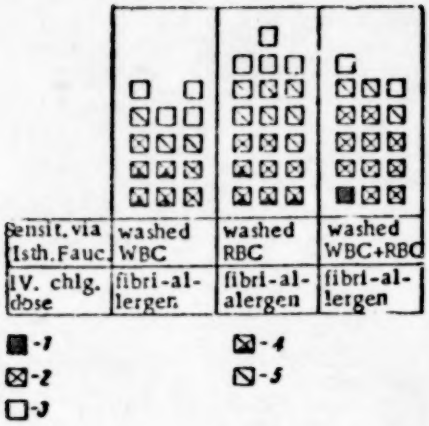

Fig. 1. Results of experiments in which guinea pigs were sensitized with washed leucocytes and erythrocytes from the blood of rheumatic patients with positive reactions to streptococcal allergen, with subsequent injection of the latter intravenously as the challenge dose.
1) Guinea pig succumbed to anaphylaxis; 2) light manifestations of anaphylaxis; 3) no reaction; 4) severe manifestations of anaphylaxis; 5) anaphylactoid manifestations.

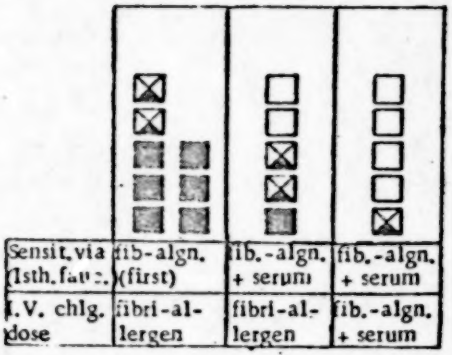

Fig. 2. Results of experiments in which guinea pigs were sensitized with filtrates of 6-day broth cultures of streptococcus and in which the specificity of streptococcal sensitizing agents was determined by the neutralization method using antianaphylactoid serum from guinea pigs which had survived anaphylaxis following sensitization and challenge doses of streptococcal allergen. Designations same as in Fig. 1.

streptococcus. Under the electron microscope, they have spherical or ovoid forms and are 200-700 millimicrons in size. This is confirmed by our investigations on prolonged incubations of inocula from the blood of rheumatic patients in whom O. V. Krasovskaya had found the formations mentioned above; under conditions of anaerobic incubation, cultures of streptococcus grow, frequently having the properties of the anaerobic microstreptococci described by A. Prevot [7]. In addition, growth of streptococcal cultures is found upon prolonged incubation in semisolid agar and on Kitt-Tarocci medium (for growing anaerobes) of blood inocula from rheumatic patients.

On the basis of the experiments conducted, we can consider that washed leucocytes and erythrocytes of rheumatic patients having a positive reaction to streptococcal allergen contain sensitizing substances which produce anaphylaxis to streptococcus in guinea pigs.

Therefore, the data obtained are a new proof of the importance of streptococci in the etiology of rheumatism.

SUMMARY

The problem was studied of the presence of adsorption of streptococcal allergens by the leucocytes and erythrocytes of the patients with rheumatism. The method consisted of sensitization of guinea pigs at the area of arci of the isthmus faucium by washed leucocytes and erythrocytes from the peripheral blood of the patients with rheumatism, with subsequent introduction of the filtrate of old cultures of streptococci to the animals as a booster dose.

As a result of these experiments anaphylaxis was obtained in 26 guinea pigs out of 49, while anaphylactoid reactions occurred in 12. Control experiments showed that the filtrates of old broth cultures of streptococci give the most pronounced sensitization in guinea pigs. These sensitizing substances are neutralized by the serum of these guinea pigs which had anaphylaxis after sensitization and introduction of the booster dose of streptococcal filtrate.

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GROWTH AND DEVELOPMENT OF BACTERIA IN CONNECTION WITH THE PHENOMENON OF BACTERIOPHAGY

COMMUNICATION 2

ELECTRON MICROSCOPE STUDY OF PHAGE FILTRATES OF MICROBES OF THE INTESTINAL GROUP

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(Received May 6, 1957. Presented by Active Member USSR Academy of Medical Sciences, N. N. Zhukov-Verezhnikov)

The interest in electron microscopic study of phage lysates and phage filtrates of bacteria has been established by very substantial circumstances.

As shown by the investigations of Raettig [4] and others, phage lysis of bacteria in a number of cases can take place without the formation of phage. With this, various formed elements of bacterial origin are found in the phage lysate. Raettig [3, 4], for example, described small formed elements, along with phage particles, measuring 12 mμ, which he named lysomes and which he thinks are formed from cellular membranes.

The data presented are significant not only in the study of the biology of bacteriophages, inasmuch as the majority of the authors indicated believe that phage particles are built of these elements, but are also extremely important for developing some idea of the morphology of filterable forms produced during phage lysis of bacteria, especially since this has hardly been studied.

Assuming that the accumulation of new experimental materials is beneficial in the one, as well as in the other instance, we conducted electron microscopic investigations of phage filtrates of microbes of the intestinal group, the results of which are the subject of the present communication.

EXPERIMENTAL METHODS

Phage lysates of strains 1321 and 1322 of Bact. coli aerogenes were filtered through asbestos filters.

Preparations for electron microscopy were prepared by placing small drops of phage filtrate on collodion films. Salt crystals were removed by dialysis for 2-2½ hours at room temperature. Finished preparations were shadowed with chromium to increase contrast.

EXPERIMENTAL RESULTS

Ten phage filtrates, checked for sterility by inoculation into meat-peptone broth and meat-peptone agar with subsequent incubation at 36° for 10 days, were subjected to electron microscopic investigation.

Despite the fact that the quantity of phage particles in the filtrate was relatively large (10^8), certain other morphological elements rather than phage particles predominated in the samples of filtrates studied.

Flat, oval elements, provisionally named "small plates," were principally encountered in the field of vision. In many instances, the "small plates" appeared as formations of bulging horse-shoe-like or irregular form, differing among themselves in size. Occasionally, well-defined outlines of fully disrupted cells were encountered in the field of vision, and also electron-dense formations, approximating phage heads in their dimensions (Fig. 1).

Fig. 1. "Small plates" and residue of lysed cell. Magnification × 36,500.

In some preparations, accumulations of essentially homogeneous oval formations, also resembling phage heads in form, were found. The presence of long shadows behind these formations indicates their considerable depth of volume (Fig. 2).

Fig. 2. Oval formations resembling phage heads in form. Magnification × 35,000.

Fig. 3. Parts of cells with dense granules. Magnification × 37,800.

In many of the preparations, the presence of larger elements, representing halves of microbial cells, was noted; some of these had dense granules (Fig. 3). We observed similar granules, which are designated as mitochondria by many authors, in earlier work in the process of vital studies of phage lysis of Bact. coli cells [1].

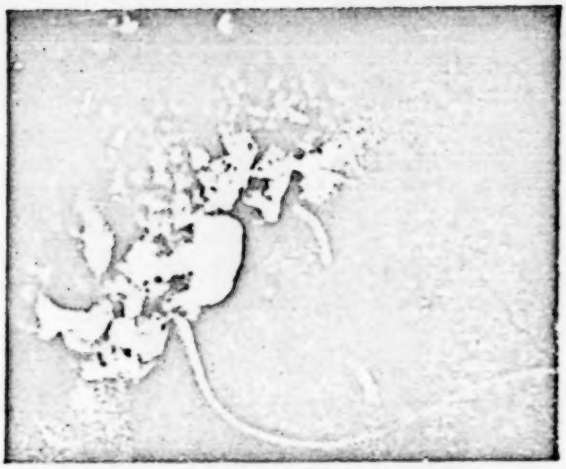

Fig. 4. Disrupted cell with flagellum. Magnification × 36,000.

In several cases, disrupted bacteria with a partially preserved flagellar apparatus were found (Fig. 4). Fragments of flagella in the form of short segments or very long sections occupying almost the entire field of vision were also found in other preparations. Finally, in single filtrates were seen little-damaged bacterial cells with clearly distinguishable internal structure but devoid of flagella (Fig. 5) and, consequently, differing somewhat in their outward appearance from normal cells of the colon bacillus.

Fig. 5. Little-damaged cell without flagella. Magnification × 40,500.

The discovery of microbial cells in the filtrates has a direct relationship to the method of filtration and the selection of filters. As is well known, in the 1930's, Chamberlain L_3 candles were principally used for filtration, excluding the passage of bacteria. In recent years, asbestos filters are chiefly used. For this reason, the results of our experiments, showing that bacteria can still be found in the filtrates in separate cases, must be taken into account in evaluating these filtrates with regard to the subject of the appearance in them of filterable forms. In any case, the assurance that the isolated secondary cultures develop from filterable forms, rather than from cells passing into the filtrate obtained with the aid of an asbestos SF filter, must involve the prior assumption of granular or some similar elements before cells are formed.

SUMMARY

The results of electronic microscopic study is presented of phage filtrates (tested for sterility) of 2 strains of Bact. coli aerogenes. Various elements of bacterial origin, different in size and form, as well as half cells with granules and intact microbe cells with or without partially or completely preserved flagellar antigen were revealed.

It is emphasized that one has to be very careful in working with the filterable forms of microbes since the cells may sometimes pass through asbestos filters "S. F." and be found in the filtrate.

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IMMUNOLOGICAL PROPERTIES OF NATIVE AND MODIFIED DESOXYRIBONUCLEOPROTEINS OF HUMAN TISSUES

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There are a number of references in the literature to the fact that modified protein does not form antibodies to the unmodified protein and that physicochemical action sharply reduces serological activity of a number of proteins [1 - 9]. However, we found no data on immunological activity of desoxyribonucleoproteins in relation to their physicochemical state or references to the degree of modification of tissues used for immunization of animals in the study of DNP.* Yet the study of DNP of organs and tissues must be very carefully conducted in view of the great lability of this protein.

In 1956 V.S. Gostev and D.G. Grigoryan developed a new method for serological study of DNP in which their fundamental property of solubility in 1 M NaCl solution and insolubility in physiological NaCl solution was utilized. Cotton fabric saturated with DNP solution in 1 M NaCl solution stably fixes a definite amount of protein when it is transferred to physiological NaCl solution.

This adsorbent, saturated with DNP solution, was used as test antigen in the serological reaction. Upon combining it with antiserums, an increase in nitrogen was observed, which was determined according to Conway. The new method permits study of the immunological activity of DNP, which is dependent not only on the species, group and organ origin, but also on the physicochemical state of the DNP.

In the present study, experiments were conducted on serums obtained by immunizing animals with "autopsy" and with operative tissues from stomach cancer and normal stomach of a human cadaver.

DNP isolated from a stomach cancer and from human thyroid gland served as test antigen in the reaction. In both cases the material was freshly frozen operative material.

A serological study was also made of DNP fixed on cotton adsorbents and dried in air at room temperature.

The study of DNP was carried out by the method of specific fixation of nitrogen on the cotton adsorbent.

Preparation of Antiserums

Antiserums were obtained by intraabdominal immunization of rabbits. Injections of increasing doses of protein were given every 24 hours for 8 days.

Animals were divided into 3 groups. The first group of rabbits was immunized with a brei of cancer tissue from human stomach, which immediately after operation was frozen with dry ice. In the future we will designate this tissue "operative tissue".

*DNP – desoxyribonucleoproteins.

The second group of rabbits was immunized with the same cancer tissue from human stomach, but this part of the tumor was frozen 24 hours after operation, having been kept at room temperature during these 24 hours. This tissue we will designate as "autopsy tissue".

The third group of rabbits was immunized with homogenate of normal stomach from a human cadaver (20-24 hours after death).

TABLE 1

Amount of Protein in Milligrams on Cotton Adsorbents Containing Native DNP from Stomach Cancer Tissue and from Human Thyroid Incubated with Serums against "Autopsy" and Operative Human Tissues

Experiment No.	DNP antigen from	A* + DNP	A+DNP+ rabbit serum	A+DNP+ serum vs. stomach cancer "autopsy" tissue	A+DNP+ serum vs. normal stomach	A+DNP+ serum vs. stomach cancer "operative" tissue	% protein increase
85	Cancer tissue from human stomach	0.157	0.580	0 560 0 612	—	0 770	37 26
91		0.297	0.595	0.542	—	0.700 0.717	29 32
92		0.210	0.962	0.980	—	1.295	32
93		0.262	1.225	1 312 1.242	—	1.802	30 46
93a		0.262	1.225	1.312 1.242	—	1.732	32 40
94		0.350	0.682	0.700	—	1.032 1.260	47 80
69		0 140	0.542	—	0.542	0.717	32
71a		0.087	0.271	—	0.262	0.385	47
72		0.073	0.233	—	0.213	0.457	100
68	Human thyroid gland	0 153	0.840	—	0.790	—	—
70		0.052	0.192	—	0.183	0.245	34
71		0.087	0.245	—	0.262	0.385	47

*A — cotton adsorbent

**Percent protein increase in samples A + DNP + serum vs. operative tissue was calculated with respect to A + DNP + serum vs. "autopsy" tissue

From Table 1 it follows that when cotton fabric saturated with DNP is incubated with normal or immune rabbit serum the amount of protein on the adsorbents increased through the fixation of both specific and nonspecific serum proteins.

The amount of protein in A + DNP with serums obtained against operative tissues differed considerably (by 26-100%) from the amount of protein obtained upon incubation of DNP with serum against "autopsy" tissue. DNP upon combination with both nonimmune rabbit serum and with serum against "autopsy" tissue from human stomach cancer fix the same amount of protein. Consequently the serum against "autopsy" tissue from stomach cancer does not contain antibodies to DNP isolated from human stomach cancer. If this were so it would give the same nitrogen increase as serum against the same tissue (except that it is operative tissue).

Analogous data were obtained with DNP isolated from human thyroid gland removed because of thyrotoxicosis. Incubation of DNP from human thyroid gland with nonimmune serum and with serum against "autopsy" tissue from a normal human cadaver gives the same results. Analogous behavior is noted in other experiments.

The next step in this work was study of serological activity of DNP of cancer tissue from human stomach adsorbed on cotton fabric and dried in air at room temperature.

Experiments were conducted similarly to the preceding ones, but with the difference that both native DNP and that modified by drying were used as test antigens (Table 2).

TABLE 2

No. of Milligrams of Protein in A+ Modified DNP of Cancer Tissue from Human Stomach upon Combination with Serums vs. "Autopsy" and Operative Tissues

Experiment No.	A+native DNP	A+modified DNP	A+DNP+normal rabbit serum	A+DNP+serum vs. "autopsy" tissue from human stomach cancer	A+DNP+serum vs. operative tissue from human stomach cancer
95	—	0.245	0.297	0.280	0.306
				0.262	0.245
					0.227
96		0.240	—	0.280	0.262
	0.098		0.227	0.282	0.542
			0.315	0.332	0.297
97	0.245	0.262	0.402	0.429	0.577

As seen from Table 2, DNP dried in air becomes serologically almost inactive. The nonspecific protein fixation, which occurred in the modified DNP with nonimmune rabbit serum, is observed also with serum against operative tissue from human stomach cancer.

Native DNP reacts in a completely different way with the same serums, while the amount of protein fixed in samples A + native DNP + serum against operative tissue from human stomach cancer was 0.542 mg, in samples A + native DNP + normal rabbit serum it was 0.227 mg. Analogous results were obtained in other experiments.

From the present study as well as literature data it follows that in study of serological properties of nucleoproteins the native state of the antigens used both for immunization and as test antigens is of critical significance. It must be assumed that the low serological activity of nucleoproteins of human and animal organs and tissues noted by many workers is due to the fact that antibodies to nucleoproteins were obtained by immunization of animals with cadaver tissue and that nucleoproteins isolated in most cases by extraction with alkali were used as test antigens.

Retention of immunological properties by DNP depends to a considerably greater extent on their native state than is the case with other proteins. Just as depolymerization and enzymic decomposition of nucleoproteins occur during autolysis, so, under conditions of partial drying of DNP modification occurs and in both cases the serological activity of DNP disappears.

Thus, a difference was found in formation of antibodies to DNP upon immunization of animals with freshly frozen operative tissues and with human "autopsy" tissues subjected to autolysis for 24 hours. The DNP isolated from cancer tissue from human stomach by the method of Mirsky and Pollister when combined with serums obtained by immunization with operative tissues are serologically active. Serum obtained by immunization of animals with "autopsy" tissues does not contain antibodies to DNP.

DNP dried in air at room temperature loses serological activity.

The physicochemical state of DNP is of fundamental significance to its immunological properties.

SUMMARY

The difference in formation of antibodies to desoxynucleoproteins in immunization of animals by freshly frozen (operative) human tissues and by "autopsy" tissues, subjected to autolysis, was demonstrated by the method of specific fixation of nitrogen on cotton adsorbent. Dried at room temperature, DNP loses its serological activity. Physico-chemical condition of DNP is of considerable significance for its immunological properties.

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SEROLOGICAL ACTIVITY OF HIGH-POLYMER DESOXYRIBONUCLEIC ACID

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The question of the serological activity of nucleic acids has been little studied and the literature contains contradictory data on this problem.

A. Menzel and M. Heidelberger [7], R. Pennel [9] and others consider bacterial nucleic acids to be serologically active and to give a specific precipitation reaction with antibacterial serums. According to data of D. Lackman, S. Mudd, J. Smolens, M. Sevag and M. Weiner [4], antiserums to nucleic acids do not give a precipitation reaction with homologous material.

Studies by Bernheimer and by Massamyne and Tokytomi [5] state that high-polymer ribonucleic acid (RNA) is serologically active, while Katsuka [3] and others report that polymeric RNA does not possess serological activity. U. Blix and C. Iland [2] assert that desoxyribonucleic acid (DNA) has serological activity.

The purpose of this study was further examination of the question of the serological activity of high-polymer DNA.

METHODS

DNA was isolated from calf thymus gland and beef liver. Thymus was frozen with dry ice immediately after slaughter and reached the laboratory in a frozen state; then it was homogenized for 1 minute in 0.14 M NaCl and 0.01 M sodium citrate to remove ribonucleoproteins according to the method of A. Mirsky and A. Pollister [8]. The precipitate was extracted in 1 M NaCl and after centrifugation reprecipitated by addition of 6 volumes of water to a concentration of 0.14 M NaCl.

The nucleoprotein threads obtained were dissolved in 1 M NaCl and the procedure was repeated several times. In order to obtain pure DNA without admixture of protein, the solution was treated with a chloroform-butanol mixture, 4:1 [10]. The procedure was repeated 18-34 times. The clear viscous DNA solution was precipitated with 2 volumes of 96% alcohol and the white threads which precipitated were dissolved in physiological solution.

All procedures were conducted at 2–4°. DNA preparations from beef liver were obtained in the same way. Phosphorus was determined in the preparations by the method of Fiske-Subbarow and nitrogen according to Conway. Molecular weight of preparations was calculated from viscosity by the method of D.M. Snitkovsky [1]. Viscosity was measured in 0.2 M NaCl in the Ostwald viscometer at 25°.

Data on chemical analysis of the DNA preparations are given below.

DNA Prepn.	Content in mg/ml		Ratio N/P	Molecular Wt.
	N	P		
No 1 (from thymus)	0.165	0.1	1.65	2 800 000
No 2 (from thymus)	0.4	0.24	1.66	5 000 000
No 3 (from thymus)	0.532	0.31	1.7	2 200 000
No 4 (from thymus)	0.0336	0.02	1 68	7 000 000
No 5 (from liver)	0.1344	0.082	1.64	6 000 000
No 6 (from liver)	0.168	0.1	1.68	7 800 000

Qualitative tests for protein were negative in all preparations.

DNA preparations upon reprecipitation with alcohol precipitated in the form of white threads readily soluble in 0.14 M NaCl, giving clear solutions of high viscosity.

The DNA preparations in 0.14 M NaCl were administered intravenously to rabbits in the amount 9-12 mg on 3 successive days. The administration was repeated during the course of 2 weeks.

Each rabbit received in all 80-110 mg DNA. Blood was taken 7-8 days after the last injection from rabbits fasted for 4 hours. The control was blood serum of the same rabbits obtained 2-3 days prior to immunization.

The serums obtained were analyzed by the complement-fixation reaction (CFR).

Antigen titer was determined in the presence of normal rabbit serum in order to eliminate anticomplementary action.

RESULTS

Experiments on the reaction of antiserums with the test antigen DNA gave the results presented in Table 1.

TABLE 1

Reaction of DNA with Antiserum (Normal Serum)

Antigen	Antiserum to DNA from thymus					Normal serum				
	1:10	1:20	1:40	1:80	1:160	1:10	1:20	1:40	1:80	1:160
DNA prepn. No.1	4+	4+	3+	2+	±	h	h	h	h	h
DNA prepn. No.2	4+	4+	3+	+	h	h	h	h	h	h

Notes. 1) The letter "h" in Tables 1-4 signifies hemolysis. 2) The control for antigens, antiserums, normal serum and complement gave hemolysis.

As seen from Table 1, DNA reacts with DNA antiserum in 1:80 dilution, giving 2+ and +, and does not react at all the normal serum. The results presented were obtained with 12 DNA antiserums from thymus and with 5 DNA antiserums from liver.

Since the DNA preparations, despite negative qualitative tests for protein, possibly contained a small admixture of protein, it was of interest to study the effect of protein on serological activity of DNA. With this purpose we checked the serological activity of preparations with respect to the extent of purification from protein.

Experimental results are given in Table 2.

Analogous results were obtained with 3 DNA preparations and with 6 antiserums to high-polymer DNA. From Table 2 it is seen that DNA with high protein content is serologically less active than DNA purified of protein.

TABLE 2

Effect of Protein on Serological Activity of DNA

Antigen	Ratio N/P	Antiserum to DNA					Normal serum				
		1:10	1:20	1:40	1:80	1:160	1:10	1:20	1:40	1:80	1:160
DNA	2	4+	3+	+	±	h	h	h	h	h	h
same	1.9	4+	3+	2+	+	h	h	h	h	h	h
"	1.73	4+	4+	3+	+	h	h	h	h	h	h
"	1.67	4+	4+	3+	+	±	h	h	h	h	h

Note. Control for antigens, antiserums, normal serum and complement gave hemolysis.

The fact that serological activity of DNA preparations does not decrease in proportion to purification from protein gives reason to assume that the serological activity is not determined by the protein. The serological reaction was conducted in a number of experiments with DNA preparations treated with crystalline trypsin. Two high-polymer DNA preparations of molecular weight 6 and 7 million were treated with trypsin for 1 hour at 37°, pH = = 8.04. In order to determine whether protein remains in DNA preparations after trypsin treatment, 15-20 mg of DNA preparations was dialyzed, the DNA precipitates after precipitation with alcohol were hydrolyzed with 6 M HCl for 28 hours with boiling and subjected to chromatographic analysis.

On chromatograms of hydrolyzates of these DNA preparations we found 4 amino acids: glycine, proline, tyrosine and leucine. Probably these are hydrolysis products of peptides and not of protein.

Results of determination of serological activity of these DNA preparations are given in Table 3.

TABLE 3

Serological Activity of DNA Preparations Treated with Trypsin

Antigen	Antiserum to DNA					Normal serum				
	1:10	1:20	1:40	1:80	1:160	1:10	1:20	1:40	1:80	1:160
DNA before trypsin treatment No. 1	4+	4+	3+	+	h	h	h	h	h	h
Same No. 2	4+	4+	2+	±	h	h	h	h	h	h
DNA after trypsin treatment No. 1	4+	4+	3+	±	h	h	h	h	h	h
Same No. 2	4+	4+	2+	±	h	h	h	h	h	h
Trypsin solution	h	h	h	h	h	h	h	h	h	h

Note. Control for antigens, antiserums, normal serum and complement gave hemolysis.

It can be seen from Table 3 that DNA treated with trypsin reacts with antiserum in 1:40 dilution, giving 3+ and 2+, i.e., exactly like the initial preparation not subjected to trypsin action. These results were obtained with 6 antiserums to DNA.

The fact that serological activity of DNA preparations does not change after trypsin treatment warrants the assumption that the serological activity is determined by the DNA itself. For further clarification of this question we conducted experiments on the effect of desoxyribonuclease (DNA-ase) on serological activity of DNA.

DNA-ase was isolated from pancreas [6] and purified from proteolytic enzymes, whose activity was determined by hemoglobin cleavage at 37° for 24 hours with subsequent determination of tyrosine at pH = 4.0, 6.22 and 8.04. The amounts of DNA-ase we used contained no proteases. The action of DNA-ase on DNA was estimated by change in viscosity of DNA solutions:

Prepn.	Viscosity	
	Before Enzyme Treatment	After Enzyme Treatment
No 1	50	10
No 2	43	11

DNA preparations not treated with enzyme after an hour in the thermostat had the same viscosity and upon reprecipitation with alcohol precipitated in the form of white threads. Preparations treated with enzyme were not precipitated by alcohol, i.e., treatment with DNA-ase led to breakdown of their structure.

Experimental results on the effect of DNA-ase on serological activity of DNA preparations are given in Table 4.

TABLE 4

Effect of DNA-ase on Serological Activity of DNA Preparations

Antigen	Antiserum to DNA					Normal serum				
	1 : 10	1 : 20	1 : 40	1 : 80	1 : 160	1 : 10	1 : 20	1 : 40	1 : 80	1 : 160
DNA before enzyme treatment No.1	4+	4+	3+	1+	±	h	h	h	h	h
Same No.2	4+	4+	3+	2+	±	h	h	h	h	h
DNA treated with enzyme No. 1	h	h	h	h	h	h	h	h	h	h
Same No.2	h	h	h	h	h	h	h	h	h	h

Note. Control for antigens, antiserums, normal serum and complement gave hemolysis.

From Table 4 it can be seen that serological activity of DNA disappears completely after 1-hour treatment with DNA-ase.

Similar results were obtained with 6 antiserums to DNA. Consequently DNA-ase, by destroying the structure of DNA, deprives it of serological activity.

It can be concluded on the basis of the material presented that high-polymer DNA possesses serological activity, giving a specific complement-fixation reaction with homologous antiserum. These properties are inherent in the DNA itself and not in possible protein admixtures, since serological activity of DNA preparations is not altered in proportion to their purification from protein or after trypsin treatment.

The serological activity is determined by the polymeric state of the DNA molecule, since depolymerization of DNA by DNA-ase deprives it of serological activity.

I am grateful to Professor V.S. Gostev for the guidance of this work.

SUMMARY

Preparations of highly polymeric DNA are serologically active and give a specific reaction of complement fixation with homologous antiserums. Serological activity is determined by DNA itself and not by the protein and treatment by trypsin has no effect on its serological activity. Serological activity of DNA is determined by its polymeric condition.

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PLASMACYTIC REACTIONS AND IMMUNOLOGICAL LAWS

COMMUNICATION I*

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The question as to the site of formation of antibodies, in spite of the many years of study, is still far from having been solved. At present a widening acceptance is developing of cytological alterations which accompany the processes of antibody production and which are denoted the "plasmacytic reaction". Associated with this has arisen the supposition that the plasma cells form the antibodies. Indeed, certain workers have observed directly the production of antibodies by plasmacytes. Thus, Fagraeus in 1948 demonstrated an accumulation of agglutinins in spleen cultures, very richly endowed with plasma cells, while in 1955 Coons, Leduc and Connolly uncovered by means of fluorescence techniques the presence of antitoxin in these cells. When compared with older concepts as to the site of antibody formation such as the reticuloendothelial and lymphoidal theories, the plasmacytic hypothesis does not express a new thought excluding the two just named. On the contrary, it really is an extension of either one or both of them and represents a finer and more contemporary analysis of the developments taking place in the lymphatic glands and spleen after the introduction of the antigen.

The aim of the present investigation was, first of all, to reproduce this phenomenon during the immunization of animals with various antigens and, in the second place, to proceed to a detailed study of the dynamics of the cytological alterations associated with the production of antibodies in the different stages of the process of immunization.

EXPERIMENTAL METHODS

Rabbits weighing 2 to 2.5 kg were immunized with adsorbed diphtheria anatoxin, the injections being made under the skin of the right shin in a dose of 1 cc this corresponding to 65 L_f. The cytological changes in the lymph glands and spleen as well as the blood antitoxin titers were determined 2, 4, 5, 6, 8, 10, 20 and 30 days after the injection. The second injection of the anatoxin was given on the 30th day while the later reinoculation was performed 6-8 months later using the same amount of anatoxin and the same site of injection. The cytological alterations and antitoxin titers were determined 2, 4, 6, 8 and 10 days after the second introduction of the antigen. Two to three rabbits were opened on each of the indicated days. Altogether, 46 immunized and 10 normal rabbits were examined.

The cytological alterations were studied from imprints taken of the right and left popliteal lymph glands and of the spleen. The rabbits were narcotized with urethane and opened and the spleen perfused with physiological saline to free it of blood, as an excess of it is reflected in a poor quality of the imprints. The perfusion

*Within our laboratory we have undertaken recently a review of previously established relationships in regard to formation of immune bodies, cytological studies being done on the lymphoid organs—the site of their formation. This is the first of a planned series of studies.

was performed by introducing the solution into the left ventricle of the heart and continued until the fluid drawn from the inferior vena cava became transparent.

The imprints made were fixed in methyl alcohol and stained with azure eosin, some being additionally colored with methyl green-pyronine. In reviewing the preparations 50 oil immersion fields were examined and the plasma cell series were counted, using the terminology of Fagraeus i.e., they were classified as transitional, immature or mature plasmacytes.

Fifty guinea pigs were immunized with heated Gartner paratyphoid vaccine, the dose being 500 million microbes and the injection being made under the skin of the right paw. They were opened under ether anesthesia. The method of preparing the imprints was the same.

EXPERIMENTAL RESULTS

In normal animals the total number of the indicated cells did not exceed 10-15 for the 50 fields examined. In all the immunized rabbits within 2 to 5 days after the introduction of the antigen there was observed a marked increase in the number of the transitory type cells, i.e., cells measuring 8-12 μ, having a regular rounded form and large, more or less fluffy, nuclei with a surrounding narrow rim of basophilic and pyroninephilic protoplasm. This cell reaction was of a rather ephemeral nature, disappearing by the 8th day. The number of mature plasmatocytes in all the preparations was quite small. The most marked cellular reactions were observed on the side on which the anatoxin was introduced. The opposite popliteal gland gave a reaction analogous in character and synchronous in time but less intense. The spleen gave negligible evidence of changes. The dynamics of the cytological changes in relation to the accumulation within the blood of the antitoxin are shown in Fig. 1.

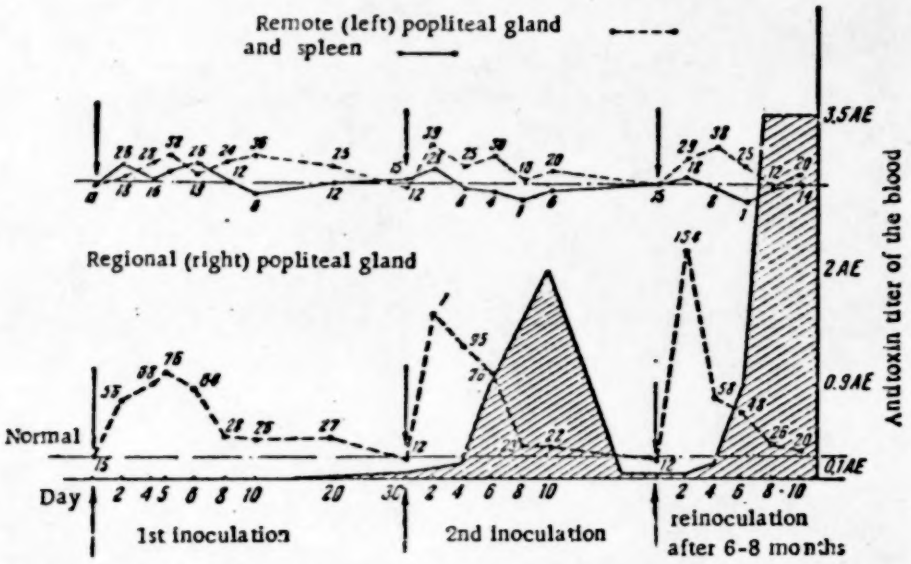

Fig. 1. The plasmacytic reaction and the production of antitoxin during immunization of rabbits with diphtheria anatoxin (average data).

As can be seen from Fig. 1, the cellular response to the second inoculation was more intense than to the first but was most vigorous to the later reinoculation. The antitoxin titer reached maximal values by the 30th day after the primary immunization (0.015 AE); by the 8th to 10th day after the secondary immunization (2AE) and by the 8th day after the later reinoculation (3.5 AE).

The results of the immunization experiments with guinea pigs are shown on Fig. 2.

Within 3 days after the introduction of the antigen, the regional lymph glands showed some increase in the number of plasmacytes, in rabbits as well as in the guinea pigs being at the expense of the less mature transitory

and juvenile cells. After some increase, by the 7th day the intensity of the cell response began to diminish, not reaching base level, however, until the 30th day. In general, the plasmacytic response to the introduction of the corpuscular antigen is characterized by a relatively low intensity and for a considerable length of time the reaction could be still demonstrated.

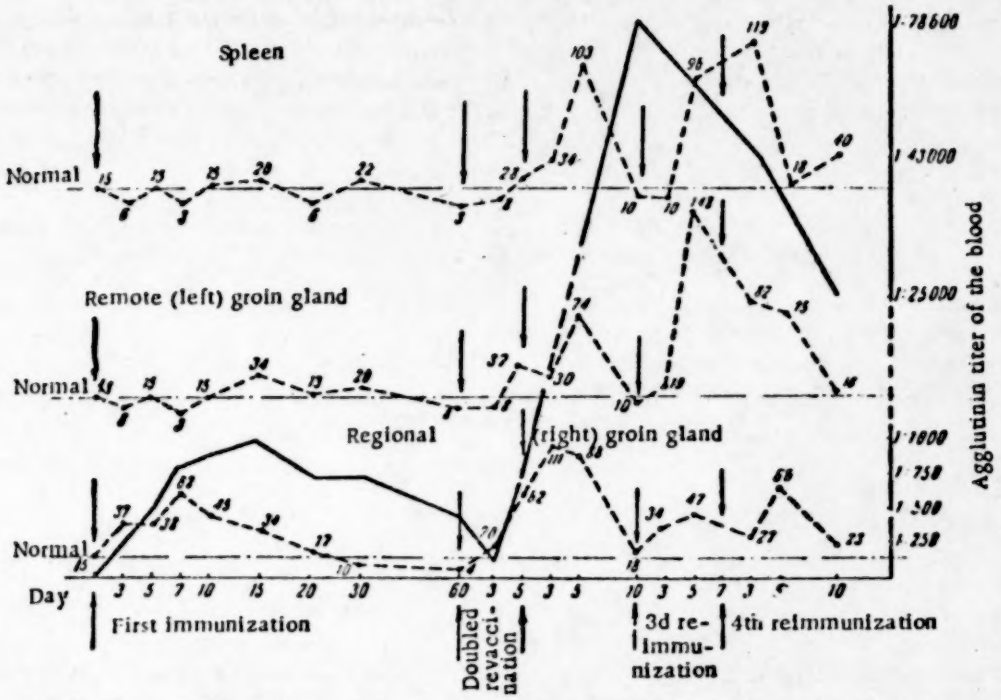

Fig. 2. Plasmacytic reaction and the production of agglutinins during immunization of guinea pigs with paratyphoid vaccine (average data).

The lymph gland in the groin on the opposite side and also the spleen showed some cellular alterations of an analogous type but lesser intensity; in time of appearance, they showed a considerable lag to the regional lymph gland changes.

The plamacytic reaction in the immunological reconstruction of the organism was studied under conditions of "doubling" reimmunization of the guinea pigs two months following the primary inoculation. The lymphatic glands and the spleen were studied before the reinoculation, on the 3rd and 5th days after the first reinoculation and on the 3rd, 5th and 10th days following the second reinoculations. As can be seen in Fig. 2, plasma cells were definitely seen to be increasing from the 5th day after the first reinoculation. By the 3rd day after the second reinoculation the plasmacytic reaction reached a maximum in the regional lymph gland, a maximum being attained in the opposite lymph gland and the spleen by the 5th day. Reinoculation produced more intense cellular responses than did the primary immunization, this result being the same as seen with the anatoxin.

The blood agglutinins increased up to the 15 . day after the primary immunization, reaching an average titer of 1:1000 and then diminishing gradually up to the 60th day. The doubled reinoculation gave a much sharper rise in the titer; the residual reading of 1:460 rose to 1:76,800 by the 10th day.

A careful analysis of the data obtained reveals that the morphological character of the reaction does not correspond completely to the usual descriptions given in the literature. The cytological alterations seen in our experiments both with anatoxin immunization and vaccine immunization demonstrated the preponderance of the early, immature forms described by Fagraeus and other authors as the beginning of the plasmacytic reaction. It is well-known that from the view-point of functional activity these cells are considered to be of the greatest

significance as an indication of the accumulation of the antibodies. However, we did not observe the further development of these forms into the mature plasma cells, i.e., the so-called "maturation" reaction did not take place, instead our experiments showed a regression of the reaction. It thus appears that the stage of mature plasma cells is not essential for the cell reaction being discussed.

The course of the plasmacytic reaction, to a considerable extent, is determined by the character of the antigen. Thus, with the primary immunization with the anatoxin the plasmacytic wave had a definite peak and ended by the 8th day, while with bacterial vaccination the intensity of the reaction was less, the wave rising and falling sharply and lasting some 20 days. The same relationships exist with revaccination. While the second introduction of the anatoxin gave maximal cytologic changes within the day, by the 5th day after introduction of vaccine the cytological alterations were still moderate, the maximal revaccination effect being attained only 5 days after the doubled revaccination. These differences may be due to the circumstance that the anatoxin, being a soluble antigen, acts directly on the lymphoidal tissues while the corpuscular antigen, before becoming a plasmacytic stimulus, has to be phagocytized and be subjected to intracellular splitting. Whatever the reasons, the fact remains there was much less response by the plasma cells to corpuscular antigens than to immunization with anatoxin.

The data obtained show that the general character of the cellular response, regardless of the antigen being used, differs from the first to the secondary challenge and seems to follow definite immunological laws, i.e., the effect of revaccination can be noted. This circumstance seems to be evidence of the close relationship the plasmacytic cytological changes being studied bear to the mechanism of antibody production.

In a search for further evidence of the association of the plasmacytic reaction with the processes of antibody formation, we attempted to determine the state of the lymphoidal elements during plasmacytic alterations occurring during manifistations of immunological inhibition as established in the laboratory of P.F. Zdrodovsky by K.T. Khalyapin, E.F. Vakarina and E.M. Golinevich as well as by A.A. Klimentova and other co-workers. It was supposed that demonstration of the inhibition of the plasmacytic reaction in the lymphoidal tissues, synchronized with immunological inhibition, on the one hand would clarify our understanding of the substrate of immunological inhibition and, on the other hand, would serve as an indirect but still substantial argument in favor of the plasmacytic hypothesis of the production of antibodies. With these goals in mind, the guinea pigs were subjected, after the doubled revaccinations (see Fig. 2) which had produced a very marked immunological response, to two more revaccinations at 7-day intervals. As in the preceding experiments, every 2-3 days three guinea pigs were opened; the spleen and the lymphatic glands of the groin were examined. At the same time, blood was drawn from the animals for serological studies.

The experiments demonstrated that the regional groin lymph gland responds much more weakly to the 3rd and 4th revaccinations, i.e., the plasmacytic reaction shows a state of fatigue. The lymph gland in the groin on the opposite side, which reacted very weakly to the primary vaccination and moderately to the doubled reimmunization, contrariwise, showed a marked response to the third introduction of the antigen and only then entered into a state of inhibition, as the fourth introduction of the antigen did not elicit a response. A very similar curve was obtained for the response of the spleen, the difference being that the marked response came with the fourth revaccination. Thus, it was found that the different lymphoid structures become involved sequentially in the immunological process, each attaining separately a state of maximum activity and then undergoing inhibition. It is quite evident that with such a state of affairs the serological blood picture cannot reflect simultaneously all the immunological processes. Actually, in the entire organism the changes described for the various organs sum up a gradual decrease and the agglutinin titer in the blood gradually falls from 1:78,600 to 1:25,000.

One method of solving the problems presented by this portion of our work would be a separate determination of the antibody titer of each organ, this being the object of our further studies. Making a general survey of our results so far, we conclude that repeated injections of an antigen may lead to inhibition of separate immunologically active organs this being accompanied by a partial decrease of their reactivity, the production of agglutinins to the last revaccination being only a third of the response to the first two revaccinations.

There is still no reason advanced for the fact of the marked lag in the blood antibody titers as compared with the changes occurring in the lymphoidal tissues seen when the rabbits were being immunized with anatoxin. Figure 1 shows that there is a disparity between the two processes expressed by a period of 20-25 days with the

primary immunization and 6-8 days with revaccination. It should be underscored that antibodies begin to increase only some time after the plasmacytic wave has ended. This fact makes it difficult to believe that the plasma cells are the direct agents producing the antibodies.

There may be two explanations for this phenomenon: either the antitoxin is formed by the plasma series cells and then gradually accumulated after some time by the producing organ, or the rise of the plasmacytic cells is not directly associated with antitoxin production but corresponds to some intermediate stage of immunogenesis in a phase preceding the obvious accumulation of the ready antitoxin. Along the indicated lines of thought, we have undertaken special studies in an attempt to determine the amount of the antibodies present in the lymph glands at various time intervals after the introduction of the antigen.

SUMMARY

Rabbits were immunized with diphtheria anatoxin. Guinea pigs were immunized with Gartner paratyphoid vaccine. Immature plasmacytic cells were shown to appear in the regional lymph glands in both instances. After a maximum had been reached, this response waned before antibodies could be demonstrated in the blood.

Repetition of the injections led to an eventual inhibition of this process so that the lymphoid organs ceased to respond with a lymphoid reaction. The regional lymph glands, the distant lymph glands and the spleen did not show a synchronization of the curves of their responses to the primary and the succeeding challenges with the antigen.

The plasmacytic reaction must be closely associated with antibody formation. The precise relationships of the various glands in the process are the object of further studies being undertaken.

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THE RELATIONSHIP BETWEEN SITE OF ANTIBODY ACTION WITHIN THE ORGANISM AND THE SITE OF ANTIBODY FORMATION (USING TETANUS ANATOXIN IMMUNIZATION AS A MODEL)

COMMUNICATION I. A NEW MODIFICATION OF THE BACHER-KRAUS METHOD AND ITS APPLICATION IN DETERMINING THE TISSUE CONTENT OF TETANUS ANTIGEN

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The goal of the present series of studies of which this is the first communication was an investigation of the topography of immunogenesis and an exploration of the laws associated with the time and space relationships governing the action of the antigen upon those structures of the entire organism sensitive to it. The necessity for such a coordinated approach arises from the circumstance that while the problems associated with the site of antibody formation (the fate of the antigen in the organism and its place of action, the dependence of the effectiveness of the immunization on the site of introduction of the antigen) are all the subjects of much study by immunologists throughout the world, these problems are being approached, as a rule, individually, without taking into due account their mutual interdependence. For this reason we took as a goal the investigation of the entire complex of the pending problems by the utilization of a single immunization model.*

Immunization with tetanus anatoxin represents in this connection a rather convenient model in view of the circumstance that the rabbits, which act as experimental animals, do not possess natural antibodies and register very clearly the revaccination effects in the absence of anaphylactoid reactions. A negative feature of this model is the difficulty of determining small amounts of the antigen without which it is impossible to follow the resorptive processes within the organism.

As is well known, tetanus anatoxin can be demonstrated with difficulty even by the use of methods as sensitive as ring precipitation, complement binding and anaphylactic testing; it is difficult to employ radioactive tracer and luminescence methods because the usual anatoxin preparations suffer from the presence of considerable amounts of impurities. This, probably, was the reason why we were unsuccessful in attempting the use of the method of hemagglutination inhibition [8].

With the aim of being able to detect minute quantities of tetanus anatoxin, we developed a new modification of the Bacher-Kraus method, the basic principle of which consists in a binding of the anatoxin by the antitoxin which is tested by subsequent addition of more toxin and injection of the mixture into mice [4, 5].

*As a basis for the presentation of the entire problem complex we employed the fact that the lymphoidal apparatus sensitizes the entire system in the process of primary immunization (the relevant material was presented at the conference of the Institute of Normal and Pathological Physiology on March 4, 1957 and will be detailed in later communications).

The changes introduced by us consist in the following.

1) The dose of the antitoxin was decreased to 0.004 AE/cc instead of the 0.2 AE/cc as usually used. The corresponding dose of the toxin was also decreased but still was kept at 2-2½ times the lethal level. The proportional decrease in the concentrations of the reacting components led to a corresponding decrease in the excess of the antitoxin which under the presenting conditions was bound by much lesser quantities of the antitoxin. It is important to note that the decrease of the concentrations apparently caused a partial dissociation of the toxin-antitoxin complex as a result of which the mixture ceased being hyper-becoming hyponeutral and so caused in mice localized tetanus and even in individual cases generalized tetanus leading to death by the 5th to 7th day with symptoms of generalized tetanus. This circumstance makes imperative the setting up of corresponding controls. When the mixture does not contain any antitoxin, all the animals perish by the 3rd to 5th day with symptoms of generalized tetanus.

2) In the usual scheme, death of the majority of mice by the 4th day is accepted as a positive result. In the modification of the Bacher-Kraus method we propose that the period of observation be lengthened to 7 days and, along with the fatalities, consideration be given to the severity of the illness of the survivors. A very essential part of the evaluation of the results obtained is a statistical treatment of the material obtained.

The experiment was performed as follows. To a single volume (0.3-1 cc) of standard antitoxin serum, diluted with physiological saline to a titer of 0.004 AE/cc, there was added an equal volume of the fluid being examined (containing known or unknown anatoxin) and one volume of physiological saline. The mixture was incubated for 45 minutes at 37° after which there was added to it one volume of tetanus toxin (we used toxin series No. 580 obtained from the Gamaleya Institute of Epidemiology and Microbiology), diluted with physiological saline to a titer of 1:50 L_t (for 0.1 AE) per 1 cc. After carefully shaking the mixture, it was again incubated at 37° for 45 minutes and then injected into the mice, each animal receiving 0.4 cc under the skin over the internal medial surface of the thigh. The experiments were performed, as a rule, with several anatoxin dilutions always using a control (the liquid volume being tested being replaced with physiological saline). The infected mice were watched for 7 days; daily and at the same hour the dead were counted and at least three separate times the clinical picture was recorded (usually on the 2nd, 4th and 7th days). Below are recorded the average results of several such experiments in which the sensitivity of the usual Bacher-Kraus method is compared with our proposed modification (in this as in all subsequent experiments we used tetanus anatoxin No. 336-4 obtained from the Gamaleya Institute of Epidemiology and Microbiology).

Comparison of the Generally Accepted Method of Titrating the Tetanus Anatoxin by the Bacher-Kraus Procedure and our Original Modification (1 L_t toxin plus 0.2 AE Antitoxin)

Titratable concentrations of anatoxin		number of mice	Results of observations by the 4th day of infection		% dying ($M \pm 2_m$)
in physiological saline dilutions	in ACE		died	survived	
1:50	1,5	12	9	3	$75 \frac{+25}{-26}$
1:75	1,0	16	8	8	50 ± 25
1:100	0,8	12	1	11	$8 \frac{+16}{-8}$
Control		12	-	12	$0 \frac{+15**}{-0}$

• and •• see next page.

An analysis of the table permits the following observations. In the first place, the modified method is at least 7 times more sensitive than the generally accepted procedure. This method is quite specific as neither the boiled tetanus nor the diphtheritic anatoxin even when used in considerably larger doses produced an effect characteristic for natural tetanus anatoxin. Also quite noticeable is the direct correlation between the dose of the anatoxin and the effect produced: mice receiving the larger dose of the antigen perished more quickly (dilutions of 1:300 and 1:500). The same correlation holds in the transition to lower doses of the anatoxin (1:1000) manifesting itself in the greater survival of the animals. Finally, when comparing the smaller doses (1:1000) with the controls, there is observed a severity of the clinical picture in the surviving animals. This makes clear the inadequacy of using only one indicator for an evaluation of the results being obtained.

Modified Method ($\frac{1}{50}$ L_t toxin + 0.004 AE antitoxin)

Titrable concentrations of anatoxin		Number of mice	Results of observations									Evaluation of the indicator (M ± 2 m)		
			Days after being infected in which animals died					Survived with symptoms of tetanus						
in physiological saline solution	• ACE •		2–3	3–4	4–5	5–6	6–7	+++	++	+	+	% dying	Prolongation of life (in days) •••	Index of intoxication •••
1 : 300	0.25	16	10	5	1	—	—	—	—	—	—	$\frac{100 \pm 0 \bullet\bullet}{-12}$	2.9 ± 0.3	7.6 ± 0.3
1 : 500	0.15	10	1	6	2	1	—	—	—	—	—	$\frac{100 \pm 0 \bullet\bullet}{-18}$	3.8 ± 0.6	6.7 ± 0.6
1 : 1000	0.08	15	—	2	—	2	1	9	1	—	—	27 ± 25	4.9 ± 1.1	3.8 ± 0.8
Control	Physiological saline	16	—	—	1	2	1	1	5	6	—	25 ± 22	5.5 ± 0.9	2.4 ± 0.9
	Boiled anatoxin 1 : 100	14	—	—	—	1	1	4	1	4	3	$\frac{14 + 20}{-14}$	6.0 ± 1.2	1.9 ± 0.9
	Diphtheria anatoxin 1 : 100	14	—	—	1	1	1	3	1	4	3	$\frac{21 + 23}{-21}$	5.5 ± 1.2	2.1 ± 1.0

• Antitoxin binding units

•• Calculated according to formula $m = \frac{100}{N+1}$ (Pomorsky, 1955)

••• Calculated with corrections for the step groupings

It is also evident that, if instead of an 1:500 dilution of anatoxin we had an unknown solution, we would be able to conclude that it contained tetanus anatoxin within limits of 0.08–0.25 ACE/cc (ACE, see footnote). In such fashion, the method being proposed permits a scale of controls (understanding by this term not only a "pure" control but also certain control anatoxin concentrations) which allows a determination of quite small concentrations not only qualitatively but also some approximately quantitative values.

An essential pre-requisite, in addition to the scale of controls, is an adequate number of animals (no less than 6-10 mice per group). In single experiments it is desirable to alter the order of infecting the various groups of the animals. The validity of the obtained data on the mortality and survival rates of the experimental animal groups is checked by the use of universally accepted statistical methods [1, 2]. As far as the indicators of the severity of the intoxication in the surviving animals are concerned (not shown in table), here there can be used along with the average values for the groups the so-called criterion of reconciliation (method x^2).

An essential and adequate basis for forming conclusions as to the variations as to the severity of the intoxication in the experimental and control groups (and thus deciding as to the presence and concentration of the anatoxin in the mixture injected into the experimental animals) lies in a statistically valid difference in any of the above indicators. However, the chosen indicator must characterize a basic portion of the experimental material and the juxtaposition of the other indicators must not contradict the conclusion made.

Naturally, separate use of the three indicators causes inconveniences in the sense that statistical analysis of the material becomes cumbersome and laborious. For this reason it seems desirable to develop some general index which could summarize the severity of intoxication existing in each group of the animals. The literature contains some attempts to solve similar problems [6, 7]. With this in mind, we attempted to devise an index with weighted values: the severity of the intoxication in the surviving animals were valued from 1 to 3, those dying on 7th day were valued as 4, those dying on 6th day were indexed as 5 and so on. The average value for the group was called the index of intoxication and the error was calculated with the usual methods. Our studies demonstrated in practice that the evaluation of the differences between the groups by means of such an index of intoxication was always confirmed when the three indices were taken separately or when other methods of evaluation were used. As, however, our present method cannot be considered generally accepted, we used it only as an auxiliary.

So far, when describing our proposed modification of the Bacher–Kraus method, we have used as a basis the data obtained by titrating the solution of tetanus anatoxin in physiological saline. It was necessary, however, to investigate the applicability of the proposed modification to titrations of fluids containing blood sera or animal tissue homogenates.

Corresponding experiments have shown that the presence in the tested fluid of rabbit blood sera or muscle homogenate did not decrease the sensitivity of the method and did not disturb the above noted correlations between the dose of the anatoxin and the severity of the intoxication of the animals (this served as a basis for the reverse decision as to the concentration of the anatoxin in the fluid being tested).

In the experiments with addition of muscle homogenate, it proved feasible to determine anatoxin concentrations of the order of 0.08 ACE/cc (this was not the limit of the sensitivity of the method), while in experiments with addition of serum anatoxin concentrations of 0.025 ACE/cc could be titrated, this being 40 times more sensitive than the usually accepted procedure. It should be noted that normal rabbit serum had a somewhat potential toxic effect. In connection with this the "control scale", when titrating for the presence of anatoxin in homogenates or sera, must contain equivalent doses of the sera or tissue homogenate of a normal animal. In addition to this, when titrating anatoxin in the presence of sera, at times (if there is found a considerable mortality in the "pure control") it becomes desirable to increase the dose of the anatoxin being added to the mixture up to 0.005 AE/cc. But, if the serum and muscle homogenate of a normal animal does not interfere with the demonstration of small amounts of anatoxin, to what extent is this true for tissue homogenates from immunized animals? In order to answer this question, tetanus anatoxin in various dilutions was added to rabbit muscle homogenate (the animal having received the day before extirpation 0.5 cc of diphtheria anatoxin (series No. 115-B-2, received from I.I. Mechnikov Institute)) after which the mixture was titrated by the method described above. As a control a mixture of normal muscle homogenate and anatoxin was used.

The results obtained demonstrate that the nonspecific or inospecific chemical alterations in the tissues which might have resulted from the addition of the antigen do not affect the titration of the anatoxin by the

proposed method. This in itself again confirms the specificity of the method and its applicability for determining the content of tetanus anatoxin in the tissues under conditions of immunization.

Thus, the more sensitive new method of titrating for tetanus anatoxin is a modification of the Bacher–Kraus procedure which permits qualitative and semi-quantitative determinations of rather small amounts of anatoxin in the presence of blood sera and rabbit muscle homogenate and for this reason this modification may be used for studying the dynamics of the antigen content of the blood and tissues of the immunized organism.

SUMMARY

A very sensitive modification of the Bacher–Kraus procedure for titrating tetanus anatoxin is proposed. This method permits a semi-quantitative titration of very small amounts of anatoxin even in the presence of blood sera and rabbit muscle homogenates. This modification is proposed for use when studying the dynamics of antigen content of the blood and tissues of the immunized organism.

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EXPERIMENTAL BIOLOGY

THE EFFECT OF EXTENSIVE RESECTION OF THE SMALL INTESTINE IN RATS ON THE PHYSIOLOGICAL REGENERATION OF THE INTESTINAL EPITHELIUM

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We [1] established that within 10 to 30 days after resecting $^1/_6$th to $^1/_7$th of the small intestine of rats there was a decrease in the number of mitoses seen in epithelium of the crypts in areas adjacent to the place of anastomosis (both proximal and distal to it) when a comparison was made with corresponding areas of the intestine in unoperated rats. At the same time, the bowel of the operated animals maintained the definite reaction of increased mitotic response to unconditional reflex food stimulators. Sixty days after the operation, the mitotic epithelial activity within the crypts of the operated rats was greater than the activity shown by the control animals or by the operated animals sacrificed 10 or 30 days after the operation. By this time, hypertrophy is apparent from the increased diameter of the bowel. Ninety days after the operation, the number of mitoses in the bowel diminishes but still remains above the level observed in the control rats.

However, it was not clear what produced the alterations in the mitotic activity within the mucosa of the bowel of the operated rats: i.e., the trauma caused by the resection or the increased functional load placed upon the remaining small intestine. Moreover, it was of interest to make a more detailed study of the destructive phase of the physiological regeneration of the small intestine after a portion of it had been resected.

In the present study we examined the physiological regeneration of the small intestine following resection of almost 50% of its length. We examined areas far removed from the anastomosis, included portions of the bowel partially excluded from the digestive process.

EXPERIMENTAL METHODS

We used 16 white rats weighing 120-180 grams. Eight were used as controls, while the other eight were operated in the manner shown in the diagram.

Mentally, we separated the intestine into four equal areas. Portion No. 2, along with its mesentery, was resected. Then the distal part of portion No. 1 was approximated to the distal part of portion No. 3, marked by us as "blind," and a side-to-side anastomosis was done. In this fashion, the blind area (No. 3) along with its mesentery, remained parallel to the proximal area (No. 1). The blind area was attached to the proximal portion by three separate sero-muscular silk sutures. On subsequent examination, we were able to prove that no food would enter the blind portion of the intestine except at the orifice directly adjacent to the anastomosis. Immediately

after the operation, the animals were taken to the vivarium and maintained there under the same conditions as the control rats. Both the control and experimental animals were sacrificed 30 days after surgery. This time interval was chosen because it was the time when the operated rats would reach the weight of the control rats of the same age. For two days before being sacrificed, the animals were given no food – only water. After the animals had been killed, their intestines were cut off from the mesenteries and totally fixed in Bouin's fluid.

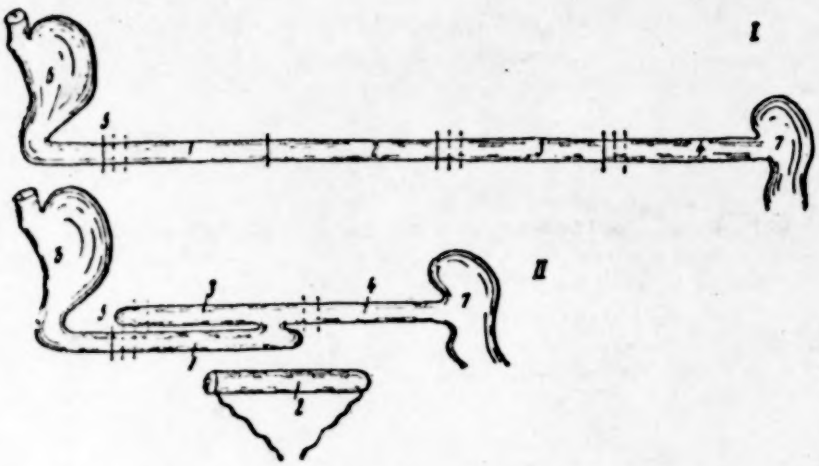

Diagram of experimental procedure. I) Before surgery; II) after surgery. 1) Proximal portion; 2) resected portion; 3) intermediate portion; 4) distal portion; 5) place where bowel passes through the mesocolon; 6) stomach; 7) cecum. Dots indicate areas taken for histologic studies.

We subjected three different areas of the intestine to morphological studies; the regions chosen were those indicated by the dotted lines of the diagram. Corresponding regions were chosen in the control animals. The material was imbedded in paraffin, longitudinal sections 7 μ thick were taken, and eosin–hematoxylin stain was used. In all the chosen areas, we counted the number of mitoses seen in 40 crypts of the sections taken longitudinally through the centers, and we then took the average number per crypt.

In addition, we qualitatively evaluated the process of epithelial sloughing and disintegration. Arbitrarily we considered it to be the first stage of sloughing either when individual cells were separating at the villous tip or when a space filled with albuminous fluid formed between the epithelium and stroma. Stage 2 was taken as being characterized by epithelial separation of a whole sheet of cells for no less than $^1/_4$ th or more than $^1/_2$ of the length of the villus. Stage 3 of sloughing was considered as having been reached when epithelial separation involved the lower half of the villus. When the epithelial sheet had already disappeared and the villus was left with only the stroma for most of its length the condition was classified as stage 4. In each rat, 100 villi were examined, each villus being categorized as pertaining to one of the 4 stages just described; then the average of the four stages of sloughing was calculated.

This qualitative evaluation of the destructive phase of physiologic regeneration is far from being a completely satisfactory method. Indeed, it is not easy to take our arbitrary division into stages as really representing the intensity of the sloughing or reflecting the various phases of the process. In addition, the end result of the secretory process depends not only on the intensity of its individual phases but also on their velocities, all these aspects of the situation being beyond the scope of our experiments at present. In spite of all this, we deemed it essential to estimate (even if only approximately) the intensity of the destructive phase of physiological regeneration in the control and experimental rats, as we did find that in the operated animals the intestinal lumen contained a larger number of sloughed cells.

EXPERIMENTAL RESULTS

The results of the mitosis count a.e shown in Table 1.

TABLE 1

The Average Number of Mitoses Per Crypt

Area	Rat No. 1	2	3	4	5	6	7	8	Average
	Operated Rats (experimental)								
Proximal	6.5	3.9	6.2	5.6	6.2	4.5	5 8	5.4	5.5
Middle	2.4	2.7	3.5	2.3	3.2	2.6	2.6	2.6	2.7
Distal	3.7	3.0	3.5	3.9	3 7	3 9	4.2	4 1	3.8
	Unoperated Rats (control)								
Proximal	4.5	3.6	3.1	3.0	2.9	3.6	3 3	4.2	3.5
Middle	3.0	2.5	3.1	3.1	3.6	2 5	2.0	2 1	2.7
Distal	2.8	2.0	2.8	2.5	2.9	2.1	2 2	2.8	2.5

As can be seen by examining this table, the average number of mitoses per crypt in the proximal portion of the bowel was 5.5 in the operated rats and 3.5 in the unoperated rats. In the middle area the number was 2.7 for both the operated and unoperated rats. The distal portion showed a greater mitotic activity in the operated animal as compared with the unoperated, the ratio being 3.8 mitoses to 2.5. The relation of the phases of the mitosis was the same for the operated and unoperated animals.

TABLE 2

Sloughing Stages in Arbitrary Units Per Each Villus

Area	Rat No. 1	2	3	4	5	6	7	8	Average
	Operated Rats (experimental)								
Proximal	3.33	2 59	3.75	1.91	1.68	2.09	3.2	2.72	2 68
Middle	1.9	1 76	1.41	2.0	0.54	0.73	1.45	0.55	1.29
Distal	3.45	1.73	2 76	2.35	1.76	0.55	0.92	1.82	1 91
	Unoperated Rats (control)								
Proximal	1.21	1.6	0.69	1.03	1.28	1.58	1.53	2.61	1.41
Middle	0 59	0.54	1.99	0.73	0.56	0.84	0.37	0.49	0.76
Distal	0.77	0.3	0.21	1.22	2.02	0.97	1.0	3.07	1.19

Thus, after partial intestinal resection, there is in the remaining bowel, an intensification of the processes characterizing physiological regeneration: in the operated rats, the proximal portion of the bowel shows a 36% increase in mitotic activity, while in the distal portion, the figure is 34% – all these values being taken as comparisons with similar areas studied in the unoperated animals. This difference in the mitotic counts is statistically valid as P equals 0.01. All this is true, however, of those portions of the intestine over which food

passes. In the middle ("blind") portion of the intestine in the operated rats, increased mitotic activity was not observed. From this it may be concluded that the alterations within the time after the operations were not due to trauma but resulted from the intestinal resection. All the rats were sacrificed under conditions such that their digestive cycles were similar, so that the increase in the number of mitoses can be regarded as a manifestation of restorative processes attempting to compensate for the resection of almost 50% of the small intestine. The fact that the number of mitoses in that middle portion of the intestine in the operated rats over which food did not pass was similar to the number seen in a comparable area in the unoperated rats compels us to suppose that the regenerative reaction of the intestine is affected directly by the passage of food through it or, in other words, that it is a function of the imposed load. Our work does not permit a decision as to whether the increase in the number of mitoses is due to increased intestinal function or due to its structural reconstruction. The possibility that both processes are proceeding simultaneously is not excluded.

The processes of epithelial sloughing and disintegration are expressed in Table 2.

Table 2 demonstrates that in the operated animals the disintegration of the epithelium proceeds more intensively, the differences being seen mainly in the proximal and distal portions of the bowel and to a much lesser degree in the middle section. The differences in the intensity of sloughing as observed between the operated and unoperated animals are statistically valid for the proximal portion. The intensification of the destructive phases in those areas where there was food passage leads us to the supposition that in the time interval after operation the processes of functional intensification predominate over those of morphological restoration. Our results on epithelial sloughing and disintegration after partial intestinal resection are in accord with the experiments of V. P. Mandzhagaladze [2] who demonstrated an increase in the solid components of intestinal secretion from an isolated portion of the intestine after the lower division of the small intestine had been resected.

SUMMARY

Eight white rats were operated, while 8 other rats were used as controls. Approximately 50% of the small intestine was resected in the 8 operated animals and, also, a blind pouch was created as shown in the diagram depicting the procedure employed. All the animals were sacrificed 30 days after surgery. The increase in the mitotic activity and the intensification of the sloughing processes seen in the intestines of the operated animals as compared with the controls was taken to be an indication of an increase in physiological regeneration. As there was no intensification seen in the blind pouch that had been constructed, it was concluded that this intensification was not due to the surgical trauma but due to the hypertrophic and compensatory processes caused by the food passing over a smaller amount of intestine remaining and therefore producing a functional overload.

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ON THE PROBLEM OF REGENERATION OF THE LUNGS IN CAUDATE AMPHIBIA

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In spite of the fact that there have been extensive investigations of the regenerative capacities of amphibia, there has been very little study on the regeneration of internal organs in these animals. In particular, the data on lung regeneration are in conflict. E. Muftich recorded lung regeneration in frogs, toads and salamanders, but not in tritons. Lung regeneration in tadpoles was obtained by K. Westphal [2]. G. Geberlein, working on axolotls came to the conclusion that they do not regenerate lungs.

The solution of the question as to whether amphibia are capable of regenerating lungs has been hampered by faulty conclusions made by a number of authors. Thus, A. Weismann [1] maintained that the internal organs are incapable of being regenerated. To confirm this opinion he performed a number of experiments; in part, he attempted to study lung regeneration in tritons.

A. Weismann performed experiments on only 3 animals; his conclusion was that triton lungs do not regenerate. However, a careful perusal of his data shows that A. Weismann had already formed prejudicial opinions before he analyzed his results. Thus, in one of his 3 cases presented in his study, there really was some apparent lung regeneration. In the triton he removed a right lung area of 2 cm dimension. Autopsy 10 months later showed the left lung to be 4.4 cm in length while the right lung measured 3.9 cm; i.e., the difference in lung lengths was smoothing out. In spite of the entirely unconvincing experimental presentation, the conclusion of A. Weismann in regard to the impossibility of the regeneration of internal organs, lungs especially, has found a widespread acceptance.

In the last few years, a whole series of Soviet authors have demonstrated the capacities for regeneration of internal organs in various vertebrate animals. In view of this, we decided to re-examine the problem of lung regeneration in caudate amphibia.

The basic portion of our work was done on the great crested newt, Triturus cristatus.

The first experimental series consisted in amputation of the right lung in adult tritons. The operation was performed in the following manner. At the base of the right anterior extremity, where there is the line dividing the transition of the dark color into the yellow, one blade of the scissors was used to penetrate the skin coverings and then make a short incision some 2-3 mm in length. The peripheral portion of the lung was then drawn up gently to the level of the anterior extremity. The amputation was performed at the place where the lung pouched out from the laryngo–tracheal juncture.

Two months following the operation, 2 tritons were sacrificed. It could be seen with the naked eye that some lung regeneration had taken place. After 9 months, 12 tritons were sacrificed. In 10 of them the observed

regeneration was such that the regenerated lungs were almost the size of the normal ones; in only 2 instances, where the amputation had apparently been too high was there no lung regeneration to be observed.

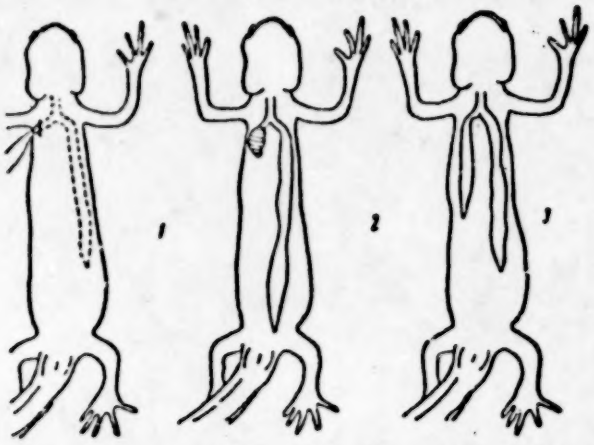

Fig. 1. Diagrammatic representation of the surgery performed and extent of lung regeneration in the triton.
1) Diagram showing place where lung was amputated; 2) three months' regeneration of the lung; 3) twelve months' regeneration of the lung.

The 3-month lung regeneration took the form of a small outpouching (Fig. 1), the color of which changed in correspondence with the cardiac rhythm. The regenerated portion was sharply demarcated from the old portion both in color and size (i.e., it was much wider). The left lung was markedly hypertrophied.

The lungs were fixed in an inflated state in Bouin's fluid and then imbedded in paraffin. Some of the sections were stained with hematoxylin – eosin, some with Weigert resorcin – fuchsin, and some according to Mallory.

Study of the histologic preparations proved that typical lung regeneration had taken place. The microscopic studies of the regenerated tissues show the difficulties of differentiating them from those of the normal (Fig. 2). In these sections, just as in normal lung, are seen the typical regions divided by the partitions. The lumen is lined with a single layer of cubical epithelium. In the walls and partitions of the regenerated organ are seen strands of smooth muscle tissue.

However, along with the indications of normal structure, typical of organ regeneration, there are some observable differences. Thus, the wall of the lung remnant is much thicker than the wall of the regenerating lung. In the regenerating lung, the transverse and longitudinal partitions are much thicker than in the normal lung and contain a much larger quantity of fibrous connective tissue as can be especially well seen on the section stained by Mallory. The partitions have vessels with larger lumens as compared with the normal lung; apparently, this vessel widening lends the red coloration to the regenerating tissue especially as the walls of the regenerating lung are thinner. The lumens within the partitions of the regenerating lung are larger than in the normal lung but their number is less. In the regenerating lung large partitions are formed much more proximally than is the case with normal lung.

The regenerating lung is more like the distal portions of a normal lung in its architecture; the proximal portion of the regenerating lung does not have short, thick partitions but forms partitions winding immediately and forming large loops, i.e., the functional component of the lung forms first. In addition, the distal portion of the regenerating lung shows a large accumulation of connective tissue elements.

Thus, our first series of experiments demonstrated that tritons have the ability to regenerate lungs. The regenerated lung was proven to be fully functional when, 6 months after the lung had been permitted to regenerate, the second normal lung was extirpated. The animals survived for a long time with two regenerated lungs.

Fig. 2. Section through regenerated lung in a triton 9 months after amputation (microphoto).

The second series of experiments was performed on the larvae of the crested triton. It is known that in the triton larvae breathing is accomplished by means of gills, the skin surface and the mucous membrane of the mouth. The lungs of the larvae have a relatively simple structure. The young larva has a lung pouch lined smoothly with loose connective tissue having occasional cells with large nuclei. Both the lung and peritoneal epithelium of the lung sac are represented by a single layer of flattened cells which are hard to tell apart from the underlying connective tissue.

The right lung was amputated in the larvae of the tritons at the level of the anterior extremity. Two larvae were sacrificed 2 months after the operation; no lung regeneration could be demonstrated. Twenty larvae which had already metamorphosed into adult tritons were sacrificed 9 months after the operation and again no lung regeneration could be demonstrated there being seen in some, at the stump only an insignificant sausage-shaped swelling.

Thus, we were unsuccessful in obtaining regeneration of lungs in triton larvae.

The third series of experiments consisted in taking a group of Amblystoma mexicanum and amputating their right lungs at the level of the anterior extremity. The operation was performed as with the adult tritons.

The animals were sacrificed at varying postoperative intervals: one, 3 months after the surgery, at which time it was found that the regenerating lung was $\frac{1}{3}$ the length of the normal one; two, 4 months after surgery, at which time the regenerating lung was $\frac{1}{2}$ the length of the normal one; two, 6 months after surgery, at which time the regenerating lung was in excess of $\frac{1}{2}$ the normal lung. Histological studies in all instances confirmed typical lung regeneration.

Thus, the results of this experimental series showed that amblystoma lungs, just as is the case with adult tritons, are capable of organ specific regeneration.

The fourth series of experiments was performed on larvae of amblystoma-axolotl, the right lung being amputated at the level of the anterior extremity. The technique of the operation was the same as with the tritons.

The operated animals were sacrificed at the following times: 1 axolotl, 2 months after surgery, at which time no lung regeneration was observed; 4 axolotls, 8 months after surgery, at which time also no regeneration could be observed.

The fifth series of experiments consisted in taking 3 axolotls and amputating their right lungs at the level of the base of the anterior extremity. In 2 other axolotls both lungs were amputated simultaneously at the level of the bases of their anterior extremities. The animals were sacrificed after 2 years and they all showed marked lung regeneration. In the cases where both lungs had been removed simultaneously the regenerated lungs looked like large red sacs reminiscent of frog lungs. In width the regenerated lungs exceeded 4 to 5 times the normal axolotl lungs.

Histologic studies showed the presence of organ-typical regeneration. There might be noted a few morphological peculiarities in the regenerated lungs as compared with the normal. The lung wall is thick. In the regenerated lung are encountered many more cylindrical epithelial cells. The epithelium carries a more transitional appearance. The crowded state of a large number of elongated cylindrical epithelial cells is testimony to the lung being in a markedly collapsed state. In the epithelium are seen oval light nuclei with, among them, some dark, rod-like nuclei; among the epithelial cells are many of the goblet type which produce the mucous entering as drops into the lung lumen.

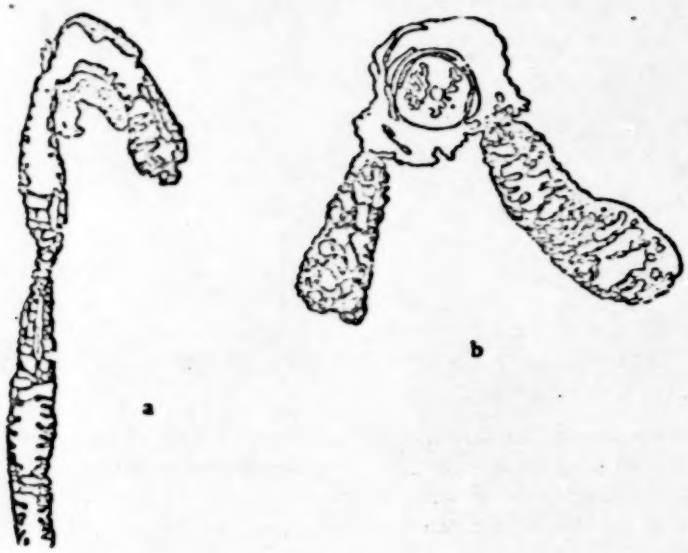

Fig. 3. Graphic reconstruction of the lungs in a triton 3 months after amputation (a) and in an axolotl 2 years after amputation (b).

In the epithelium are many, mainly lymphoidal migratory elements, also encountered are some polymorph-nucleated leucocytes. In places, are seen spaces filled with hematogenous elements, this being especially true in the distal end of the regenerating lung. Individual capillaries are packed with lymphoidal elements.

The blood vessel net is more markedly developed in the regenerating lung and it indents the lung lumen. Such a marked vascularity brings to mind the thought that we are dealing with a compensatory adaptation of the blood capillary network. Figure 3 shows graphically the reconstructed lung in a triton and an axolotl.

In addition to the peculiarities observed in regenerating lung of axolotl as compared with the normal lung, there may be noted some differences in lung regeneration in an axolotl as compared with amblystoma.

In amblystoma regenerating lungs do not attain the width while being longer (up to $^2/_3$ that of the normal lung), at a time when the axolotl regenerating lung is shorter.

Summarizing our results we can say that in adult tailed amphibia regeneration of organ-typical, normally functioning lungs takes place. Triton larvae did not show lung regeneration at all, while in axolotls lungs regenerate more slowly than in amblystoma.

The experimental data obtained indicate the great importance of the age of the animals and also the significance of functional differences producing regeneration or else failing to do so. To a certain extent, our data help to explain the contradictions in the views of various authors as to the possibility of obtaining lung regeneration among amphibia.

SUMMARY

Contrary to previously expressed opinions, a series of experiments, performed on adult crested newts, Triturus cristatus, demonstrated that entire lungs could be amputated and then made to regenerate into fully functional new organs.

The importance of the age factors and the functional state of the lungs at the time of amputation was demonstrated by the failure to obtain regeneration in triton larvae.

Amblystoma mexicanum and axolotl were similarly tested in other experimental series. The axolotl was shown to regenerate lungs but more slowly than amblystoma.

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MORPHOLOGY AND PATHOMORPHOLOGY

THROMBOCYTES UNDER THE ELECTRON MICROSCOPE

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Lately, hematologists are being attracted more and more to the use of the electron microscope in their studies of the formed elements of the blood and bone marrow. The study of the finer cell structures in normal and in pathological states may help refine our understanding of some phases of the pathogenesis of aplastic and hypoplastic anemias, leucoses and other diseases of the hematological system. In particular, the morphology of thrombocytes and an investigation of their finer structure is of special interest as they play such a well known role in blood coagulation.

The first data on the electron microscopic structure of blood platelets are associated with the work of Wolpers and Rusca [10]. Their studies were followed by the works of Bessis [4], Porter and Hawn [8], Alexandrowicz and Blicharski [3], Braunsteiner, Fellinger and Pakesch [6] and many others. The data accumulated by all these authors permit not only formation of an opinion on the structure of the thrombocytes but also unfold some of their significance in the complex process of blood coagulation.

In the present communication, which is our first on the work we have done using the electron microscope in analyzing the cells of the blood, we offer the results of our study of the thrombocytes in 20 healthy people, 10 patients suffering with leucoses and 12 patients ill with aplastic and hypoplastic anemias. We chose this particular group of patients as hemorrhagic diathesis is frequently the most important feature of their entire clinical picture.

EXPERIMENTAL METHODS

The preparations for investigation were made in the following fashion: the heparinized blood was centrifuged for 10 minutes at 1000 rpm, then the plasma was carefully pipetted off and centrifuged again for 20 minutes at 1500 rpm. The thrombocytes, which were separated by this method, were placed on special copper nets which had been first covered with a colloidal membrane. After remaining in a thermostat at 37° for 10-15 minutes, the preparations were rinsed with Ringer's and then fixed in a 2% solution of osmic acid and finally rinsed with distilled water.

Thrombocyte preparations vary according to the time elapsed between the drawing of the blood and the preparation for electron microscope studies, so that we always employed blood prepared 30-35 minutes after it had been drawn.

The thrombocytes were examined under an electron microscope EM-3 of native construction giving a magnification of 7,000 times.

EXPERIMENTAL RESULTS

According to our observations, which coincide with the findings in the literature (G. M. Abdullaev [1], F. Alexandrowicz [3], M. Bessis [4], G. Braunsteiner [6], G. I. Roskin [2] and others), the thrombocytes of healthy people when examined under the electron microscope present many polymorphic formations with innumerable pseudopodia and outgrowths.

In the thrombocytes of donor bloods there is easily distinguished a peripheral zone called the hyalomere and a central portion called the granulomere (Fig. 1).

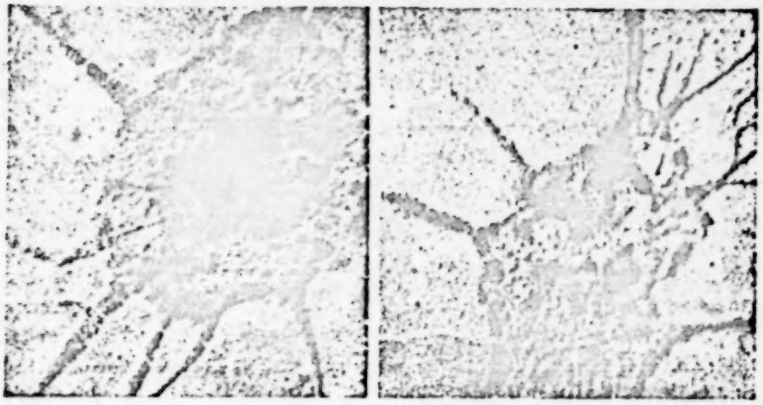

Fig. 1. Electron microscopic pictures of blood thrombocytes taken from healthy people. Magnification 7,000 times.

The hyalomere is not homogenous, structurally speaking, and consists of numerous fibrils intertwined with each other (M. Bessis [5]). This fibrillar network protrudes at the periphery of the thrombocyte forming outgrowths and protrusions of variable dimensions; from tiny serrations to pseudopodia (Fig. 2). The pseudopodia have different thicknesses and lengths, although the thickness is always greatest at the point of attachment to the body of the thrombocyte. Also encountered are pseudopodia which branch further and then intertwine with each other. The number of pseudopods present in individual thrombocytes varied, in our studies being within the limits of 2 to 12.

The thrombocytic granulomere consists of numerous grain-like granules, packed closely together in specific places and seeming to form a single conglomerate. Usually, the granulomere assumes a central position (see Fig. 1) but it may be eccentric (see Fig. 2). However, we saw individual instances when the granulomere granules were distributed evenly over almost the entire surface of the platelet.

Concerning the roles played by the hyalomere and the granulomere in the complex blood clotting process, there exists a diversity of opinion in the literature. Most authorities (M. Bessis [4], G. Braunsteiner [6] and others) believe that, when the platelets disintegrate, from the hyalomere which has a fibrillar reticular structure the microsomal grains which are the carriers of thrombokinase are liberated. These authors believe that the granulomere represents the center from which the fibrin threads radiate.

The other point of view is held by F. Alexandrowicz and I. Blicharski [3]. They consider that the disintegration of the hyalomere frees the granulomere and as a result of this the thrombocytes liberate the lipoidal factor which participates in the formation of thromboplastin into the surrounding plasma. This is the precondition for the beginning of the blood coagulation process. In the opinion of these authors, the hyalomere participates in the retraction of the blood clot.

In the light of new data published lately on the blood clotting mechanism (P. Owren [7], M. Stefanini and V. Damechek [9]), we are inclined to prefer the second view point.

Fig. 2. Electron microscopic pictures of thrombocytes taken from patients suffering with aplastic (a) and hypoplastic (b) anemias. Magnification 7,000 times.

Examination of the thrombocytes, obtained from patients ill with aplastic and hypoplastic anemias, by means of the electron microscope reveals that the thrombocytes of these patients have a morphology distinct from the morphology of the thrombocytes obtained from healthy people. The first difference lies in greater morphological uniformity of the platelets. The numerous serrations and pseudopodia observed in donor thrombocytes are either altogether absent or else much less marked (Fig. 2).

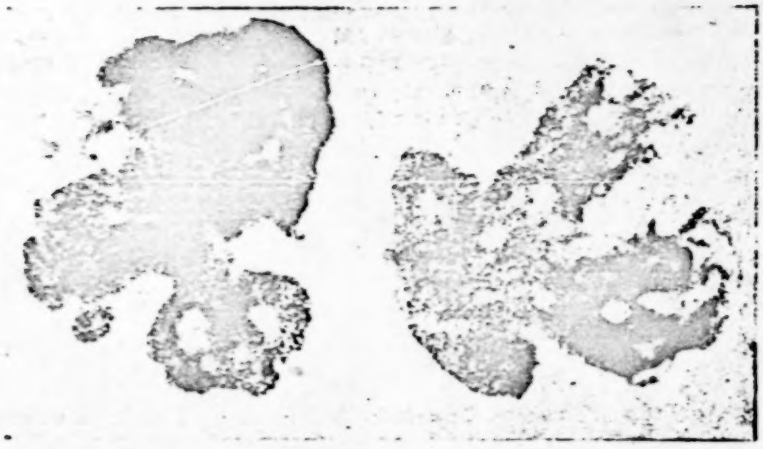

Fig. 3. Electron microscopic picture of the thrombocytes in the blood of a patient ill with leucosis. Magnification 7,000 times.

The surface of the thrombocytes obtained from these patients is smoother, frequently shows no outgrowths or serrations, and may possess only 1 or 2 pseudopods which are usually quite short. Similar to the thrombocytes

of healthy people, the thrombocytes of hypoplastic anemia patients have 2 zones, namely, granulomere and hyalomere. However, in the case of the patients' thrombocytes, the boundaries between these zones tend to become erased. Within the platelets is seen a considerable number of sharply demarcated vacuoles of various forms and sizes; this may possibly be an indication of degenerative alterations.

Distinct morphological changes are also to be found when the thrombocytes of patients suffering with various forms of leucosis are examined. In these patients the degenerative alterations within the platelets are quite marked. Vacuolization, absence of serrations and pseudopods, as well as the almost total inability to determine the boundaries between the hyalomere and granulomere within the thrombocytes of leukemic patients appear to be indicative of marked abnormalities (Fig. 3). These findings agree with the findings in the literature [2].

The present paucity of observations makes it difficult to correlate the precise morphological alterations in the structure of the thrombocytes with the exact clinical course taken by the disease. There is the over all impression that in specimens from the patients having severe hemorrhagic manifestations, there are demonstrated in the electron microscope the sharpest deviations from normal in the structure of their thrombocytes.

The electron microscopic picture presented by the thrombocytes of patients ill with leucoses, aplastic and hypoplastic anemias, in our opinion, may be of aid in understanding the mechanism underlying the development of hemorrhagic tendencies in these diseases.

SUMMARY

The electron microscope was used to study human thrombocytes at magnification of 7,000 times. Thrombocytes taken from healthy donors were compared with thrombocytes obtained from patients having leukemias and various aplastic and hypoplastic anemias.

The details of the granulomere and hyalomere structures were compared. It was pointed out that they may have a bearing on the hemorrhagic tendencies these diseases show clinically.

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METHODS

AN ELECTRONIC REFLEX METER

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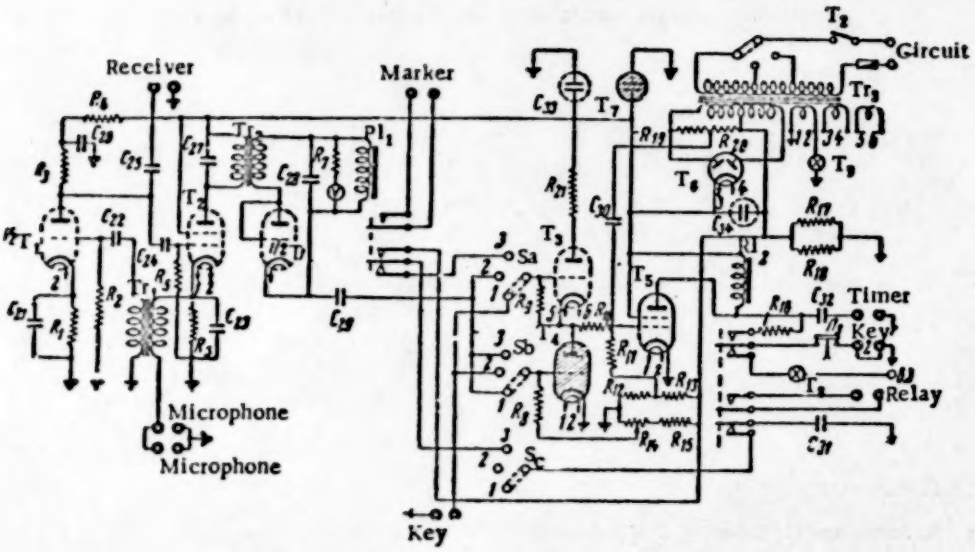
The latest achievements of radio electronics open up vistas for the development of more exact and varied methods for the measurement of reflexes. The present communication presents a short description of a perfected variation of the electronic reflex meter TKhP-56. The instrument had been constructed by us and P. N. Karpenko in 1951 [1,2] and has been perfected at present with the participation of P.I.Rumyantseva. In 1956-1957 experimental commercial models of this apparatus were subjected to prolonged testing in a number of the institutes of the Acad. Sci. USSR and Acad. Med. Sci. USSR and received favorable comment. The technical committee of the Ministry of Health USSR has approved the apparatus and recommended that it go into regular production.



Schematic of the electronic reflex meter.
R_1-R_{20}) Resistances; C_{21}-C_{32}) condensers; Tr_1) microphone transformer; Tr_2) output transformer; Tr_3) power transformer; Rl_1, Rl_2) electromagnetic relays; S_c-S_c) wafer switch; S_2) cut off switch; S_3) push-button "start 3"; T_2-T_9) tubes.

The electronic reflex meter is an "inertia-less" instrument for investigating various conditioned and unconditioned reflex responses to direct and verbal stimuli. In part the electronic reflex meter permits the study in man and animals of verbal (voice) and movement reactions in various combinations, as well as study of respiratory, tendon, defensive and some other reflexes.

The concrete experimental situations made possible by the electronic reflex meter are best analyzed in relation to a study of conditioned movement and voice reactions. Depending on the experimental problem, the investigator presents to the subject some signal: direct (light, sound, mechanical to skin, electric stimulus, and others) or verbal. Simultaneously with the appearance of the signal, the electric timer cuts in automatically. The subject, having absorbed the signal, reacts to it, depending on the experimental conditions, be it a response by movement or word. The very beginning of the movement or word (of any intensity beginning with a whisper) has to stop the timer. This in itself measures the latent period of the response to the stimulus. Depending on the experimental situation, the electric timer may be activated not only at the start but also at the completion of the movement or word. The presence of two electric timers makes possible the simultaneous measurement of both the movement and voice reactions to the same signal.

The apparatus receives alternating current from the line at 127/220 volts. The diagram of the apparatus is shown in the figure.

The basic elements of the apparatus are: the electronic relay, connected in circuit with the timer, the regulating tube and thyratron, this latter being used to direct the electronic relay.*

The receipt of the verbal or direct conditioned signal is associated with an action upon the circuit of the control tubes; this results in the opening of the electronic relay and the beginning of current passing through the timer. The subject, having assimilated the signal, reacts to it with a movement or word, thus activating the thyratron circuit; as a result, the electronic relay shuts off and the timer stops.

The intensity of the verbal signal or the verbal reaction is registered with the aid of a microphone (laryngo-phone) and amplifier by means of an electric indicator. The circuit of the timer becomes activated at the end of the verbal signal by the use of a condenser which becomes charged at the beginning of the word and becomes connected with the circuit of the control tube after the word has been pronounced. The electronic reflex meter is built to take the electric timer PB-52 which is obtainable commercially but other timers may be modified from other systems.

The possibility of employing this instrument under variable situations is attained by the switching of the special commutator. The sensitivity can be regulated. The electric timer may be switched in or out not only with a word but also by the start or end of a sigh, swallowing and other activities. When necessary, objective registration by the apparatus can include addition to the forward panel of an oscillograph or an electromagnetic marker. In the presence of a portable transmitter, the electronic reflex meter can operate as a wireless receiver and the distance between the experimenter and subject can be varied (wireless radio variant).

The electronic reflex meter makes possible a whole series of experimental psycho-neurological experimental procedures as well as such methods of studying conditioned reflexes as speech-motor (in animals: voice), electrodefensive, blinking, swallowing and others.

The apparatus is easy to transport (dimensions 32 x 17 x 12 cm). It is easy to use, reliable and not complicated in construction.

SUMMARY

The author describes an electronic reflex meter which extends the present methods used for the study of various conditioned and unconditioned reflexes.

The diagram shows the electronic relays, connected in circuit with an electric second meter, the control tube and the thyratron circuits.

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* There exists a variant of this apparatus in which, instead of a thyratron, a trigger device is employed.

TECHNIQUE OF PREPARATION OF PYROGENIC POLYSACCHARIDE FROM PROTEUS VULGARIS

A. A. Bodarev and M. V. Svyatukhin

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Reports in the foreign press have been regularly published in recent years on the special effect of pyrogenic polysaccharides of bacteria on the course of pathological processes. Polysaccharide preparations of Proteus vulgaris and Bacillus pyocyaneus, released under the name Pyromen, have been particularly studied.

According to these reports, injections of pyrogenic polysaccharides accelerate regeneration of injured nerve fibers [1], promote regeneration of nerve fibers in sectioned spinal cord [8] and partial restoration of conduction [5], normalize blood circulation in injured tissues and prevent necrosis of the frostbitten rabbit ear [6], promote repair processes, lessen scarring and even aid in resolution of newly formed cartilage in the burned ear [2, 7]. Wexler and Tryzynski [6, 7] and Randolph and Rollins [4] reported successful use of Pyromen in treating allergic diseases.

These data as well as many years of clinical experience in fever therapy indicate that the attention of both clinicians and experimenters should be directed to study of the action of pyrogenic polysaccharides on various pathological processes. However, obtaining pure nontoxic preparations involves a number of difficulties. Hence, we decided to report our work on obtaining such a preparation from Proteus vulgaris.

The method we used does not involve original procedures. But after a series of unsuccessful attempts we succeeded in selecting the conditions and a combination of successive procedures permitting preparation of pyrogenic polysaccharide nontoxic in doses exceeding the pyrogenic dose hundred of times.

From a multitude of proposed methods we chose the precipitation by acid acetone and subsequent treatment with phenol according to Palmer and Gerlow [3]. In order to work with minimal volumes of the initial bacterial suspension, washings from an agar culture were used and not a culture in liquid medium.

In order to avoid admixture of endotoxin, which it is difficult later to get rid of, it was decided to avoid use of the ordinarily recommended procedures, such as shaking the bacterial suspension and acid hydrolysis. We observed all necessary precautions in the work for preventing contamination of the preparation by extraneous pyrogenic substances.

The sequence of work was as follows.

1. Wash off a 48-hour culture of Proteus with physiological solution. Use each portion of the latter over again to wash the culture into several small vessels.

2. Centrifuge. Filter centrifugate through a bacteriological candle.

3. Pour filtrate into 10 volumes of acid acetone (in all cases the acetone contains 0.4% by volume of glacial acetic acid).

4. The following day pour off acetone and dissolve precipitate in 360 ml water (in all cases use twice-distilled water distilled with potassium permanganate and sterilized in the autoclave immediately after distillation).

5. Precipitate again with 10 volumes of acid acetone.

6. After 24 hours dissolve precipitate in 150 ml water. Dialyze in colloidal sacs at about 0° against frequently changed distilled water for 5 days (to almost complete decolorization of solution and cessation of coloring of water).

7. Precipitate with an equal volume of 95% ethyl alcohol. Centrifuge.

8. Precipitate the clear supernatant with 10 volumes of acid acetone. Centrifuge.

9. Twice dissolve precipitate in a small amount of hot water and treat with shaking for a day with 6 volumes of crystalline phenol.

10. Decant phenol. Wash precipitate thrice with acetone and dry under vacuum.

11. Dissolve in a small amount of hot water. Centrifuge to remove insoluble particles. Precipitate supernatant with 10 volumes of acid acetone.

12. Centrifuge. Wash precipitate thrice with small portions of 95° ethyl alcohol and dry under vacuum.

A white powder of light gray tint is obtained, which gives a sharp Molisch reaction and no biuret reaction. From washings from two hundred 0.7-1 liter flasks of square section 0.3258 g of the preparation was obtained. Rabbit tests showed that the preparation in amounts of 1-500 γ per kilogram is not toxic on parenteral injection.

It produces a sharp temperature reaction in a dose of 1 γ per kilogram.

Results of testing pyrogenic activity of the preparation are given in the table.

Results of Testing Pyrogenic Activity of Preparation

Date of experiment	Rabbit No.	Rabbit wt. (in g)	Dose (γ/kg)	Temper. before injection of prepn.	Temperature after injection of prepn.				
					after 30 min	after 1 hour	after 3 hours	after 5 hours	after 7 hrs.
13/III 1956	227	2870	Physiological soln.	38.3°	38.5°	38.6°	38.7°	38.8°	38.2°
	224	2860	"	38.7°	38.5°	38.7°	38.8°	38.6°	39.4°
	225	2860	5.0	38.5°	39.0°	40.5°	41.4°	40.9°	40.5°
	222	3130	5.0	38.7°	39.4°	40.4°	40.9°	39.8°	38.8°
	164	3200	20.0	38.6°	39.3°	40.3°	41.2°	40.0°	40.0°
	201	2630	20.0	38.9°	39.6°	40.6°	40.7°	39.7°	40.0°
	211	2950	100.0	38.8°	39.0°	39.8°	40.5°	40.1°	39.4°
	170	2680	100.0	39.0°	39.9	40.2°	40.4°	40.3	39.9°
8/IV 1956	206	2920	Physiological soln.	38.0°	38.2°	38.0°	38.0°	38.1°	—
	214	3150		38.8°	38.9°	38.8°	38.9°	38.8°	—
	234	3130	0.1	39.1°	39.0°	39.5°	39.1°	39.0°	—
	220	2030	0.1	38.4°	38.6°	39.3°	39.0°	38.8°	—
	219	2940	1.0	38.8°	39.3°	40.3°	40.2°	38.7°	—
	210	3030	1.0	38.3°	38.6°	39.4°	39.3°	38.7°	—
	232	2940	200.0	38.2°	39.4°	39.4°	40.7°	40.1°	39.0°
	276	2900	200.0	38.7°	39.8°	39.9°	39.7°	39.7°	39.5°
	277	2860	500.0	38.5°	39.3°	39.6°	38.8°	39.0°	—
	258	2790	500.0	38.2°	39.4°	38.9°	38.1°	38.1°	—

Rabbits that received the preparation in amounts of 200 and 500 γ per kilogram were given a second parenteral injection of the preparation in the same amount after 2 weeks. No symptoms of anaphylactic shock were observed.

Thus, by means of simple technique available to practically every bacteriological laboratory, a Proteus vulgaris polysaccharide preparation can be obtained, which is nontoxic and nonanaphylactogenic in amounts exceeding the pyrogenic dose hundreds of times.

SUMMARY

A method of preparation of pyrogenic polysaccharide from B. proteus vulgaris is presented. At the basis of this method lies the precipitation of the washings of agar culture by acidified acetone from the noncellular filtrate with the following treatment by phenol (Palmer and Gerlow method). The preparation which is thus obtained is nontoxic and nonanaphylactogenic for rabbits in the doses up to 500 γ per kilogram of body weight. This method is simple and may be used in every bacteriological laboratory.

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QUANTITATIVE REACTION OF SPECIFIC NITROGEN FIXATION BY PROTEIN ANTIGENS ADSORBED ON DERMATOL AND PAPER

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Of much importance to serology is the use of quantitative methods of study, which express the results of a specific reaction not by plus signs but by exact, readily reproducible figures. Among such quantitative methods one of the most important is the determination of immunological affinity between antigen and antibody with respect to the amount of protein nitrogen stably bound in the products of a specific reaction [3, 4, 7, 9, 10, 11, 12]. Dermatol in particular has been proposed [1, 2, 6] as adsorbent of antigenic substances.

Attempts were made in our laboratory to develop a quantitative method for serological study of antigens adsorbed on inert particles. A. K. Saakov (1953) showed that dermatol particles after adsorbing antigens upon reaction with appropriate antiserum give a specific nitrogen increase in the agglutinate form.

The purpose of the present paper is further development of methods for quantitative study of the serological reaction of adsorbed antigens.

In the first part of the study we reproduced data obtained by Saakov in his work on dermatol-antigen in order to evaluate it and to attempt to find more convenient procedures for quantitative serology.

EXPERIMENTAL METHODS

Preparation of antigens adsorbed on dermatol:

a) Preparation of dermatol fractions. 200 mg dermatol was suspended in 30 ml distilled water. The suspension was fractionated by centrifuging in a hand centrifuge. Three fractions of dermatol particles were obtained:

Fraction I (heavy) – precipitate obtained after 10 minutes of centrifuging.
Fraction II (medium)– precipitate obtained after 15 minutes of centrifuging.
Fraction III (light) – precipitate obtained after 15 minutes of centrifuging supernatant fluid from Fraction II.

Fractions I and III were discarded. Fraction II was dried and kept in a weighing bottle. We used the medium dermatol fraction for experiment, since it was found in preliminary experiments that compared to the other fractions it has the greatest adsorptive properties with respect to protein antigens.

b) Preparation of antigen. From an aqueous-saline extract of cancer tissue of human stomach, prepared by grinding with 10 volumes of physiological solution, after centrifuging the globulin fraction was isolated by

* Russian trade name.

means of 33% saturation with ammonium sulfate. After dialysis the protein content of the globulin solution was determined by Conway's method.

c) Adsorption of protein on dermatol particles. Batches of fraction II of dermatol (60 mg) were placed in centrifuge tubes, to each tube was added 5 ml of a solution of the globulins of cancer tissue of human stomach, containing about 1 mg protein in 1 ml. Tubes were stoppered with rubber stoppers and shaken in the cold every 2-3 minutes for 1 hour, then placed in the refrigerator overnight. On the following day shaking of tubes was repeated every 2-3 minutes in the cold for 1 hour. Washing samples free of nonadsorbed protein was carried out strictly in the cold by repeated centrifuging until a clear centrifugate was obtained.

d) Antiserum was obtained by intraabdominal immunization of rabbits with a brei of cancer tissue of human stomach.

e) Reaction of specific protein increase in dermatol agglutinate. To dermatol-antigen was added 3 ml of antiserum (1 : 10 dilution) in the experimental sample and 3 ml of normal rabbit serum (1 : 10 dilution) in the control. Experimental and control each consisted of three parallel samples. All samples were placed in the thermostat at 37° for 2 hours, shaken every 10 minutes and placed in the refrigerator overnight. The next day all samples were washed in the cold with physiological solution by repeated centrifugation, then transferred quantitatively to Kjeldahl flasks for combustion with concentrated sulfuric acid. The results obtained are given in Table 1.

TABLE 1

Protein Increase (in mg) With Respect to Nitrogen in Dermatol Agglutinate In Presence of Normal and of Immune Serum

Expt. No.	D-A + normal rabbit serum	Antiserum	D-A + antiserum	Specific protein increase
1	1.575	№ 1831	1.6275	0.0525
2	1.575	№ 1672	1.8725	0.2975
3	1.9425	№ 1831	2.7325	0.79
4	1.9425	№ 1672	2.2225	0.28
5	1.8025	№ 1382	2.2175	0.415
6	1.8025	№ 1672	2.3275	0.525
7	1.61	№ 1387	2.24	0.63
8	1.61	№ 1672	1.75	0.14
9	2.1	№ 1387	2.415	0.315
10	2.1	№ 1880	2 607	0 507
11	1.229	№ 1775	1.721	0.492
12	1 229	№ 1951	1.47	0.241
13	1.00625	№ 1231	1.27625	0.27
14	1.00625	№ 1951	1.16375	0.1575
15	1.4	№ 1387	2.131	0 731
16	1.19	№ 1572	1.505	0.315
17	0 69125	№ 1672	0.76125	0.07
18	1 705	№ 1382	1.8025	0.0975
19	1.3475	№ 1672	1.8025	0.455

Note: D-A – dermatol-antigen (globulin fraction of cancer tissue of human stomach); antiserum – serum of rabbit immunized with brei of cancer tissue of human stomach.

Results of 19 experiments are shown in Table 1, from which is seen the marked increase in protein nitrogen in the dermatol-antigen through specific fixation of antiserum proteins by the adsorbed antigen.

However, treatment of dermatol-antigen with normal serum not containing the antibodies also leads to marked nitrogen increase in the dermatol precipitate through nonspecific fixation of serum proteins. In order to determine the amount of specific fixation of antiserum proteins from the total increase in protein nitrogen it is necessary to deduct the protein nitrogen bound to dermatol-antigen in the control with normal serum.

The quantitative agglutination reaction of dermatol particles which have adsorbed antigen has a number of substantial disadvantages. The method is very laborious, it requires a powerful centrifuge for washing the dermatol precipitate and leads to great expenditure of work and time. The quantitative transfer of the dermatol precipitate to Kjeldahl flasks for combustion is a great inconvenience. Dermatol-antigen cannot be used in the complement-fixation reaction in view of its strong anticomplementary action. These substantial defects of the dermatol agglutination method led us to search for other adsorbents for antigens, permitting readier and more rapid quantitative study of serological reactions.

We selected chromatographic paper, cotton fabric and cotton wool as adsorbents.

These materials fix proteins stably if the latter are coupled with bis-diazotized benzidine or dianisidine. This recommendation was made to us by Professor A. I. Korolev for which we express our sincere gratitude.

Protein antigens, fixed to a paper surface, in the presence of antiserum are able to fix stably the serum proteins, producing specific increase of protein nitrogen.

1. Preparation of azo-antigens fixed to paper. Dianisidine ($C_{14}H_{16}O_2N_2$, molecular weight 244.3) in the amount 61 mg is dissolved in 50 ml distilled water and diazotized with cooling in an acid medium by fractional addition in 0.5 ml amounts of 0.1 N $NaNO_2$ solution. HCl is added in excess, since the diazonium salt formed upon reducing acidity of the medium may react with unreacted amine with formation of secondary products. The diazotization is controlled by testing on starch-iodine paper. A positive reaction on starch-iodine paper, whose stable and distinct blue color from 1-2 drops of sample does not disappear during 6-8 minutes, indicates presence of excess sodium nitrite in the medium and consequently completion of diazotization.

The next step is coupling the diazodianisidine obtained with protein.

Aqueous-saline extracts of human organs – cancerous stomach, normal stomach, spleen and human serum globulins were used in our experiments.

The protein solution was made alkaline by addition of a half volume of 5% Na_2CO_3 solution and the freshly prepared diazodianisidine (diazonium salt) was added with constant stirring in the cold in divided portions of 0.5 ml. A positive reaction with N-acid indicates completion of the coupling reaction. The azo-protein obtained is adsorbed on strips of chromatographic paper, dried at room temperature and after washing with tap water for 5-6 hours again dried at room temperature. The diazodianisidine was introduced into the protein molecule in order to assure stable fixation of protein to the chromatographic paper. Azo-protein can also be fixed to cotton fabric and cotton wool.

2. Reaction of specific protein increase. In order to study specific activity of paper azo-antigen we developed a new serological reaction – the reaction of specific fixation of antiserum protein by azo-protein adsorbed on paper [5, 8].

To a batch of paper azo-antigen (6 mg) on a glass slide with a depression, 0.1 ml of appropriate undiluted antiserum is added. As control 0.1 ml of undiluted serum not containing antibodies to the given antigen is added to a similar batch of antigen and 0.1 ml physiological solution to another. The samples on glass slides in a moist chamber are placed in a thermostat at 37° and after 2-hour incubation are put in the refrigerator overnight. Then the pieces of paper azo-antigen are washed with 3 successive portions of 10 ml of cold physiological solution for 5 minutes each. The washed samples are subjected to combustion with sulfuric acid and their protein content is determined by Conway's method.

In Table 2 is given a typical protocol of one experiment.

It is seen from Table 2 that the paper antigen, an azo-protein from globulins of normal human serum, adsorbed on chromatographic paper, gives a distinct specific increase of protein upon incubation with serum against human tissues. Together with specific adsorption there occurs in this case nonspecific adsorption, which must also be considered in calculating the specific increase.

In spite of a number of advantages of the paper azo-antigens and the simplicity of the method of determining nitrogen increase (the antigen retains activity for several months, can be readily transported and has no appreciable anticomplementary properties) we cannot avoid noting one very substantial factor. In the process of coupling protein with diazodianisidine the protein undergoes quite strong chemical action, which cannot avoid being reflected in its immunological properties. There is no doubt that the protein is to a certain extent denatured.

TABLE 2

Protein Increase (in mg) in Azo-Antigens Adsorbed on Chromatographic Paper

Paper azo-antigen	Amt. of protein in adsorbed antigen	Anti-serum	Normal rabbit serum	Physiological solution	Amt. of protein after incubation	Specific protein increase
weight in mg		in ml			in mg	
6	0.07875	0.1	–	–	0.2625	0.13125
6	0.07875	–	0.1	–	0.13125	–
6	0.07875	–	–	0.1	0.07875	–

Note: Paper azo-antigen – azo-globulins of human serum; antiserum – serum against human tissues (undiluted).

Now we have developed a new procedure for preparing a so-called native antigen fixed to paper which is without the defects of the azo-protein method.

EXPERIMENTAL RESULTS

Into an aqueous-saline extract of human tissue (prepared by grinding tissue in 10 volumes of physiological solution) strips of chromatographic paper were immersed for 10-15 minutes. The paper after saturation with protein solution was dried in air at room temperature, washed with tap water for 3-4 hours and again dried at room temperature. The reaction of protein nitrogen fixation was performed as with the paper azo-antigens described above.

TABLE 3

Protein Increase (in mg) in Native Antigens Fixed to Chromatographic Paper

Paper antigen	Amt. of protein in paper antigen	Anti-serum	Normal rabbit serum	Physiological solution	Amt. of protein after incubation	Specific protein increase
weight in mg		in ml			in mg	
20	0.06125	0.2	–	–	0.21875	0.105
20	–	–	0.2	–	0.11375	–
20	–	–	–	0.2	0.06125	–

Note: Paper antigen – aqueous-saline extract of cancer tissue of human stomach, adsorbed on chromatographic paper; antiserum – serum against brei of operative cancer tissue of human stomach.

In Table 3 are given the results of determination of serological activity by the method of specific increase in protein nitrogen of native proteins fixed to chromatographic paper. The data, in the form of a typical protocol, indicate the pronounced serological activity of the new type of fixed antigens we obtained.

The final form of the method of fixing protein to paper adsorbent proposed in this report is very simple and practicable. Upon saturating paper with protein solution, drying it, washing and again drying, a serologically

active antigen is obtained, which invariably gives the reaction of fixing protein nitrogen of antiserum. In fixing proteins to paper with simple drying at room temperature the protein studied is considerably less denatured than when diazodianisidine is introduced into the molecule.

Paper antigens obtained by the method of coupling with diazo-compounds, as well as native paper antigens, retain serological activity for several months. Paper antigens are being used successfully by us at the present time for extraction of general species antibodies with the purpose of obtaining highly specific serums.

The reaction of protein fixation by antigen adsorbed on paper is a new serological reaction. It cannot be called either agglutination, precipitation or complement-fixation. It is a reaction of a special sort. The antigen adsorbed to the paper or cotton surface is able by binding with antibody to fix stably antiserum proteins. The reaction is specific and expresses quantitatively the degree of immunological affinity in a given antigen-antibody system.

SUMMARY

A specific addition of protein nitrogen was obtained in the agglutinate of dermatol particles, covered by antigen. Protein combined with azo-dye with bis-diazotized dianisidine gives stable sorption on the surface of the paper, cotton material or cotton wool. Such paper azo-antigen gives a pronounced specific addition of protein nitrogen which comes from the proteins of the antiserum. Analogous data were obtained with native paper antigens, secured by simple drying of the protein solution in which chromatographic paper is soaked. Reaction of specific fixation of the protein nitrogen on the surface of the paper antigen represents an easily performed new serological reaction in immunology. This reaction gives a numerical value of quantitative characteristics of the immunological antigen – antibody relation.

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